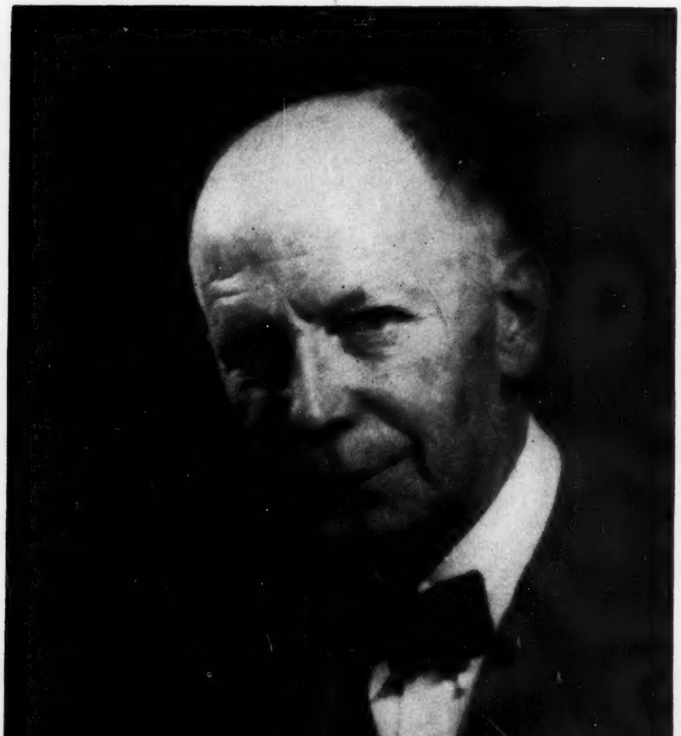
DIABETES

The Journal of the American Diabetes Association



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Third Lilly Conference on Carbutamide

Indianapolis, Indiana

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Active work on the sulfonylurea compounds was undertaken in the United States in 1955. Following preliminary clinical studies confirming the German experience, the First Lilly Conference was held in December of that year. The available data were presented to a group of experienced investigators in the field and plans made to study various aspects of the problem. The Second Lilly Conference was convened in April 1956, when the progress of these early investigators was summarized. It seemed evident at that time that the sulfonylureas were not only effective in many diabetics but also apparently devoid of serious toxic manifestations. Extension of the trials to include a broad base of clinical material was then undertaken, since treatment of significant numbers of patients is always essential to thorough evaluation of any new product.

The symposium published in this issue of DIABETES represents the accumulation of still further data presented at the Third Lilly Conference on Carbutamide held in Indianapolis in September 1956, and includes the tape recording of discussion following each group of reports. A survey of clinical experience in the broad trial which included about 10,000 patients was presented, providing a more accurate estimate of the potential hazards of side effects, these ranging around 5.3 per cent. There were a few deaths in treated cases in which the drug may have been implicated although conclusive evidence was lacking. In view of these side effects broad clinical trial of carbutamide was curtailed and an announcement of its suspension pending further investigations was sent to clinical investigators by Eli Lilly and Company.

There are still many facets of the action of hypoglycemic drugs which remain to be explored. The question of whether or not the hypoglycemia produced represents a potentially toxic manifestation of their action cannot yet be decided. There appear to be two main types of side effects: those associated with hypersensitivity or allergic manifestations (including effects on the blood cells), and those influencing the metabolism of the liver. The two may not go hand-in-hand, and some compounds may prove safe enough to use clinically. Actually the toxicity of carbutamide is comparatively quite low. Certainly it would be no deterrent to treatment of any serious temporary illness, e.g., pneumonia, nor would it be considered serious if no other safe treatment were available for diabetes. It is a nice question to contemplate—how much toxicity can be tolerated in a drug used in the management of a disease which may extend over an ordinary lifetime?

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Toxicological Studies on Carbutamide

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In 1942, Janbon[1] first observed the hypoglycemic action of certain sulfonamides when he was investigating the antibacterial action of sulfanilamido-isopropylthiadiazoles. Loubatières[2] showed that the compound was inactive in depancreatized dogs and postulated that it brought about hypoglycemia in normal animals by stimulating insulin secretion. In 1955, Achelis and Hardebeck[3] and others [4, 5] reported on the hypoglycemic action of *p*-aminophenylsulfonyl butyl carbamide, carbutamide or BZ-55, in normal rabbits, in normal man and in some diabetic patients. Inasmuch as carbutamide has continued to lower the blood sugar of certain diabetics, long-term toxicity studies have been pursued.

METHODS

Acute toxicity. Albino mice, weighing 14 to 18 gm., were starved overnight prior to injection. For intravenous studies, a 10 per cent solution was prepared with a minimum of Na_2CO_3 (*p*H 8.2). A 20 per cent suspension in sesame oil was used for intraperitoneal injection and a 20 per cent suspension in 5 per cent acacia for the subcutaneous and oral tests. Starved albino rats, weighing 80 to 120 gm., were employed for the acute toxicity studies, a 20 per cent solution in Na_2CO_3 being used intravenously and a 40 per cent suspension in acacia for oral administration. All animals were observed for one week and deaths or any signs of toxicity recorded. The median lethal doses (LD_{50}) were calculated by the method of Bliss.[6]

Blood analyses. Blood sugar values were determined by the Hagedorn-Jensen method [7] and the blood carbutamide concentrations by a modified Bratton-Marshall procedure.[8]

Subacute toxicity. Seven albino New Zealand rabbits, weighing between 2.2 and 2.7 kg., were given daily doses of 1,000 mg. per kg. by stomach tube until each succumbed. A freshly prepared 25 per cent suspension in 5 per cent acacia was employed.

Chronic toxicity: rats. Essentially the same method reported previously from these laboratories was used.[9] Fifty-five female Harlan rats, weighing between 70 and 90 gm., were studied. Ten rats were fed a diet containing 1 per cent carbutamide and ten a diet with 2 per cent carbutamide; five rats on normal diet served as controls. Later, three groups of ten rats each were placed on 0.5 per cent, 0.25 per cent and normal diet respectively.

Chronic toxicity: dogs. Eighteen dogs, weighing from 5.3 to 10.0 kg., received daily doses of 12.5 to 500 mg. per kg. by capsule. Blood samples for various analyses were taken from the jugular vein and urine was obtained by catheterization.

Chronic toxicity: monkeys. A 15 per cent suspension of carbutamide was administered daily to eleven Rhesus monkeys by stomach tube. Blood samples for counts and analyses were taken from the cubital vein. All animals on chronic studies were submitted for necropsy at death or after sacrifice.

RESULTS

Acute toxicity. The doses used, number of deaths, and the calculated LD_{50}'s after intravenous, subcutaneous, intraperitoneal and oral administration to albino mice are shown in table 1. Similar data obtained from rats following intravenous and oral doses are also included.

After intravenous injections, mice had convulsions within one minute, followed by prostration and death in three to five minutes. Intraperitoneal median lethal doses of carbutamide produced deaths, after twenty-four to forty-eight hours, whereas larger doses killed in eighteen hours. Some deaths were recorded within twenty-four hours after subcutaneous or oral administration, while others occurred two to four days later. Rats responded similarly to mice to intravenous injections, and died from two to five days after oral administration. Some reduction in the blood sugar values was found; deaths were not attributable solely to hypoglycemia, as blood sugar values in rats were 104 to 122 mg. per 100 cc. just before death. Blood carbutamide concentrations were greater than 100 mg. per 100 cc. at the same time.

Subacute toxicity: rabbits. Three rabbits succumbed after three daily doses of 1,000 mg. per kg. Necropsy revealed hemopneumothorax caused by mechanical damage during administration. The other four rabbits survived 13, 38, 48 and 68 doses, respectively. All gained weight during the drug regimen, but died from pulmonary edema provoked by the entrance of the stomach tube into the trachea. Blood sugar values fell from

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around 100 mg. per 100 cc. to about 75 or 85 following the dose and returned to normal before the next dose. Blood carbutamide rose to around 40 mg. per 100 cc. about two hours after dosing and returned to a trough of less than 1 mg. per 100 cc. just before the next dose. To a large extent, the drug was present in the blood in the free form. In some rabbits conjugated carbutamide was found, but it rarely represented more than 20 per cent of the total present in the blood.

Chronic toxicity: rats. The effects on the growth curves of rats fed diets containing 0.25, 0.5 and 1.0 per cent of carbutamide are compared with those of control rats in figure 1. At the end of ten months all rats on the 0.25 per cent diet survived and remained on test. Two fed the 0.5 per cent diet died after injuries received while being restrained in a holder during blood sampling. No abnormalities were seen at autopsy. Two rats in the 1.0 per cent group died after 33 and 121 days, the former from malnutrition and central necrosis of the liver, the latter from malnutrition, bronchiectasis and crystalluria. Eight remained on test in the 0.5 per cent group after ten months and eight were still on test in 1.0 per cent group after fourteen months. Rats fed the diet containing 2 per cent carbutamide died after 29, 75, 84, 103, 117, 118, 131, 161, 191 and 205 days. Malnutrition was apparent in all and three showed crystalluria as well. Slight hypertrophy of the thyroid was also evident in about half of these animals.

Blood carbutamide and blood sugar values, determined on pooled samples from five rats in each group, are found in table 2. The carbutamide concentrations were greater than 70 mg. per 100 cc. blood in rats on the 2 per cent diet. No marked hypoglycemia has been found in rats in the chronic studies.

Chronic toxicity: dogs. Carbutamide has proved to be more toxic to dogs than to other species. The results are summarized in table 3. All dogs that received either 12.5 or 25 mg. per kg. daily remain on test; those on higher doses have succumbed. Necropsies revealed degranulation of the beta cells of the pancreas, hypertrophy of the thyroid and erosions of the gastric mucosa in the three dogs on 50 mg. per kg. Daily doses of 100 mg. per kg. produced the same pathological changes and in addition caused fatty metamorphosis of the liver in two of the three. Higher doses produced quicker deaths with marked hypertrophy of the thyroids and bleeding into the gastrointestinal tract.

Biweekly blood and urine analyses were made. They showed a fall in erythrocyte and leukocyte counts and a reduction in hematocrit and hemoglobin values in dogs on toxic doses. No changes in clot retraction or

TABLE 1

Acute toxicity of carbutamide in mice and rats

Species	Mode of administration	Dose gm. per kg.	No. died / No. used	$LD_{50}\pm$S.E. gm. per kg.
Mouse	I.V.	1.25	0/10	1.92±0.08
		1.60	2/10	
		2.0	5/10	
	I.P.	2.0	2/10	2.10±0.04
		2.25	9/10	
		2.50	10/10	
	S.C.	2.0	0/10	2.64±0.11
		2.5	4/10	
		3.0	8/10	
	Oral	2.75	0/10	3.46±0.16
		3.30	6/10	
		4.0	7/10	
Rat	I.V.	0.8	0/5	0.98±0.04
		1.0	3/5	
		1.25	5/5	
	Oral	8.0	0/5	10.31±0.70
		10.0	3/5	
		12.5	4/5	

TABLE 2

Blood carbutamide and blood sugar levels in rats after being fed diets for nine months

Per cent drug in diet	Carbutamide mg. per 100 cc.	Blood sugar mg. per 100 cc.
0.25	10	97
0.5	15	104
1.0	32	124
Controls	—	114

TABLE 3

Chronic toxicity in dogs of daily oral carbutamide

Daily dose	Status	Effect on body weight	Blood level Trough	Blood level Peak
mg. per kg.			mg. per 100 cc.	mg. per 100 cc.
12.5	3 survive after 157 doses	Gain		
25	3 survive after 157 doses	Gain	4-6	10-14
50	Deaths after 28, 43 and 73 doses	Loss	4-12 25-46 (Terminal)	
100	Deaths after 32, 50 and 92 doses	Loss	20-40	69-100
250	Deaths after 22, 24 and 24 doses	Loss	39-60	95-111
500	Deaths after 8, 10 and 10 doses	Loss	—	—

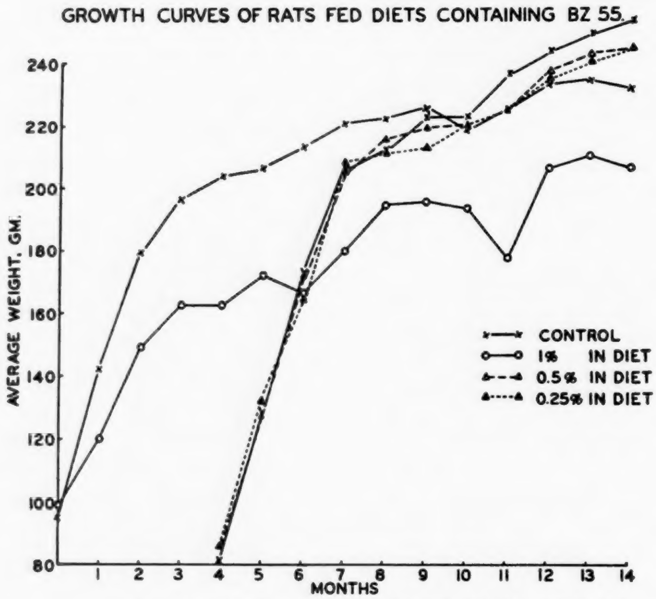

FIG. 1. Growth in rats being fed normal diet containing various per cents of BZ-55.

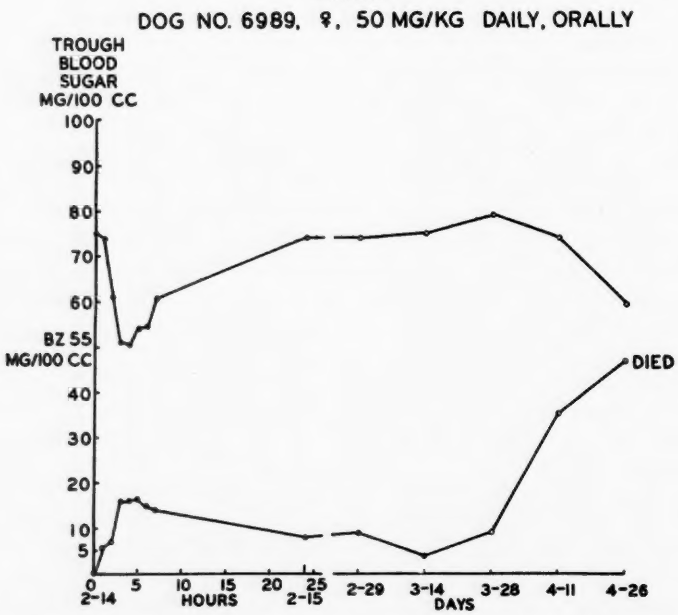

FIG. 2. Typical blood BZ-55 and blood sugar curves in a dog.

whole blood clotting times were noted. In some instances nonprotein nitrogen values rose just before death. No proteinuria or glycosuria was found.

Blood carbutamide and blood sugar curves were determined at repeated intervals. Those found in dog No. 6989 after the first dose on February 14 are plotted in figure 2. Repeated doses were well tolerated through March 28 and trough values of carbutamide — i.e., values found just before the next dose—remained low. Thereafter, however, carbutamide trough concentrations rose and remained within the toxic range during the two weeks preceding death. Figure 3 indicates that the blood sugar continued to fall after daily doses; the response obtained after the 157th dose tended to be similar to that which followed the initial dose.

Chronic toxicity: *monkeys*. Rhesus monkeys tolerated

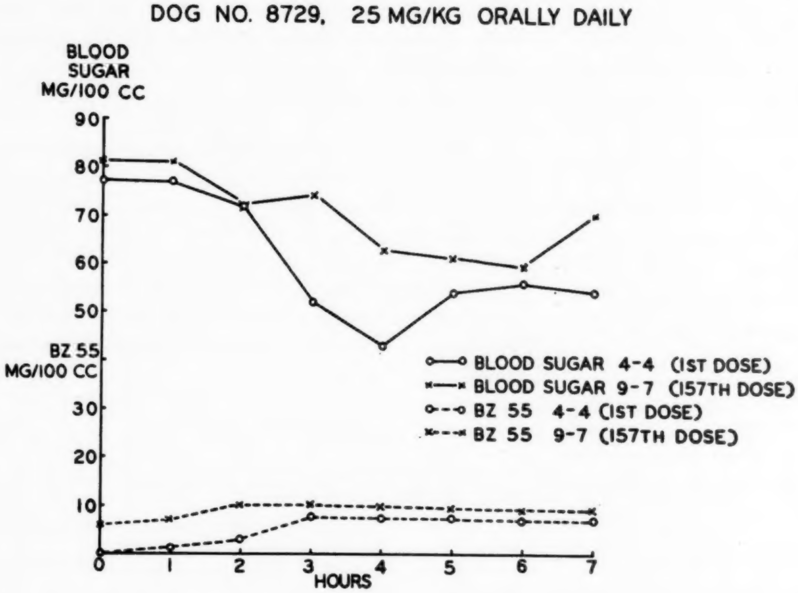

FIG. 3. Blood sugar curve and blood value of BZ-55 in response to the 157th dose of the drug in a dog.

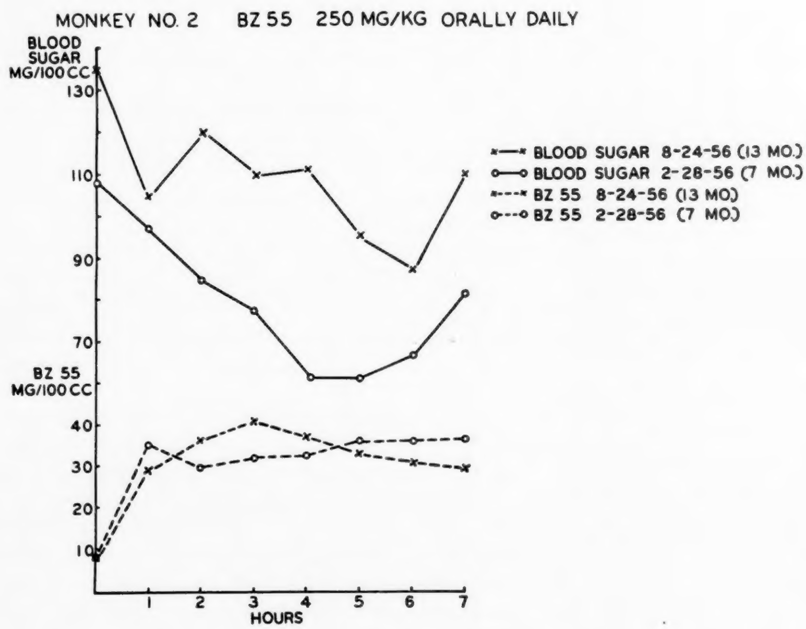

FIG. 4. Response of blood sugar and drug concentration in a monkey after a long daily dosage of BZ-55.

large doses of carbutamide for an extended period of time (table 4). Seven are alive and remain on test. Four monkeys succumbed after repeated doses of 500 mg. per kg. daily. Pulmonary edema and hydrothorax were found in all with no other visceral damage evident.

The typical blood sugar response in monkeys is shown in figure 4, the fall obtained after thirteen months of daily treatments being about as great as that found six months earlier. Blood carbutamide curves also determined after seven and thirteen months were practically superimposable, which shows a lack of accumulation of carbutamide in these monkeys.

TABLE 4
Chronic toxicity in monkeys of daily oral carbutamide

Daily dose	Status	Effect on body weight	Blood level Trough	Peak
mg. per kg.			mg. per 100 cc.	mg. per 100 cc.
100	3 survive after 203 doses	Gain	2.0-6.0	18-29
250	3 survive after 287 doses and 1 after 421 doses	Minimal	6-18	40-50
500	Deaths after 8, 9, 25 and 73 doses	Loss	45	82

Frequent blood analyses revealed no marked changes in erythrocyte, leukocyte or differential counts, or hematocrit and hemoglobin levels, or whole blood clotting and clot retraction times, or in nonprotein nitrogen values.

DISCUSSION

Subacute and chronic toxicity studies have demonstrated marked differences among various species. Rabbits cleared carbutamide from the blood stream rapidly so that trough levels remained very low after repeated doses of 1,000 mg. per kg. Monkeys tolerated daily doses as large as 250 mg. per kg. for from 41 to 60 weeks. Here again the blood carbutamide trough levels were low and little or no conjugated carbutamide was found in the blood. Dogs, however, succumbed after doses of 50 mg. per kg. daily. As long as the blood carbutamide trough level remained below 10 mg. per 100 cc. the dogs gained weight and appeared normal. When the concentrations began to rise and clearance was reduced, toxic signs developed and death followed. Marshall et al.[10] showed that dogs do not acetylate sulfa drugs as do rabbits and men. Although, carbutamide is not conjugated to a large extent by rabbits, monkeys or man, it would appear that the detoxification mechanism in the dog differs from that in those species and that, when the rate of absorption exceeds the rate of destruction, toxic effects are produced.

SUMMARY

1. Carbutamide had a low order of toxicity in laboratory animals following single doses by various routes.

2. Rats, rabbits and monkeys tolerated large daily doses for an extended period of time. Dogs, however, survived doses only one-tenth the size of those given monkeys.

3. Rabbits and monkeys cleared carbutamide from the blood stream so that trough values remained low. Dogs appeared to lose the ability to detoxify carbutamide quickly.

4. Rabbits, dogs and monkeys continued to show a fall in blood sugar after repeated doses.

5. No visceral or hematopoietic damage attributable to the drug was found in rabbits or monkeys. Rats on high concentrations showed some crystalluria, malnutrition and hypertrophy of the thyroid. Dogs that received toxic doses had degranulation of the beta cells of the pancreas, hypertrophy of the thyroid and erosions of the gastric mucosa with a reduction in hematocrit and hemoglobin values, and a lowering of erythrocyte and leukocyte counts.

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COMMENT

DR. PAUL HARRIS (*Indianapolis*): Pathologic changes in the animals listed in Dr. Anderson's tables have not been impressive. Three rabbits died as a result of trauma following three daily doses of BZ-55; four others died of pulmonary edema following more prolonged daily dosage. No changes were seen in the other viscera.

Two rats given BZ-55 in the diet at a level of 0.5 per cent died of accidental causes and showed no lesions attributable to the drug. Two rats receiving the compound at a level of 1 per cent in the diet died; one showed malnutrition and necrosis of some cells in the center of nearly every liver lobule; the other also showed malnutrition and some crystals in the kidney pelvis Malnutrition was apparent in all of ten rats that died after receiving the drug in the diet at a level of 2

per cent; three showed crystalluria. Microscopic examination of tissues was not deemed profitable in three rats because of advanced post-mortem change; one rat died after twenty-nine days with necrosis of some cells near the center of every liver lobule, and another had unilateral pyelitis with necrosis of the apex of the renal pyramid.

Two of three dogs given daily doses of 50 mg. per kg. showed minute erosions of the gastric mucosa. All showed hypertrophy of the thyroid gland. One of the three given doses of 100 mg. per kg. had several gastric ulcers, and all showed thyroid hypertrophy. Two also showed fatty metamorphosis of the liver. The beta cells of the pancreatic islands of four of these six dogs showed partial degranulation. This change is one that I have seen in animals other than those included in Dr. Anderson's tables, but it has been quite variable in extent and percentage incidence.

Four monkeys died at various intervals after the daily administration of 500 mg. per kg. doses was begun. All showed pulmonary edema and hydrothorax. Three also showed fat vacuolization of liver cells, but the significance of this is not clear, since this is frequently true of monkey livers.

Hypoglycemic Sulfonylureas in Various Types of Experimental Diabetes

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Recent articles on the capacity of certain sulfonylureas to lower the level of blood glucose have reviewed the early observations and summarized the first experiences with their use in man.[1-5] A special number of the *Canadian Medical Association Journal*[6] presents nineteen articles on the sulfonylureas and includes studies on two depancreatized and one Houssay dogs. One of the first questions concerning these compounds has been: Does their action depend upon the presence of insulin? In other words, will they act in the absence of insulin? They are apparently ineffective in depancreatized dogs[6, 7] and man.[6, 8] The following observations support this conclusion and extend the conditions in which the drugs have been tested.

METHODS

Alloxan diabetic rats. Rats of the Wistar strain of both sexes weighing 140 to 180 gm. were made diabetic by the intraperitoneal administration of 175 mg. per kg. of alloxan. One to two weeks later animals which excreted several grams of sugar in the urine daily, while eating an adequate constant diet, were selected for the experiments. They were kept in metabolism cages and fed a weighed amount of Purina dog chow daily. The urinary glucose was determined[9] daily and the blood sugar[10] at times. When given orally for periods of several days, the sulfonylurea was added to the weighed amount of food, which had been pulverized and moistened to make a thick paste.

Cats have been tested by observing the effect of a single intraperitoneal dose of a sulfonylurea on the blood sugar during the subsequent six to eight hours. Some of these tests were made under sodium pentobarbital anesthesia and some in unanesthetized animals. The results were not affected by anesthesia. Such tests were performed in normal, hypophysectomized, depancreatized, and Houssay (hypophysectomized and depancreatized) animals. There was one death from barbiturate anesthesia in the first Houssay cat tested so that anesthesia was not used in any hypophysectomized animals thereafter. All cats were kept in metabolism cages, were fed weighed amounts of fresh horse meat daily, and in both types of depancreatized animals urinary glucose was determined daily and the presence of ketone bodies tested by the nitroprusside method.

The complete removal of pancreas and pituitary was determined by physiological criteria and by autopsy. The principal criteria were: for pancreatectomy, the amount of glucose excreted during fasting, ketonuria, and at

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autopsy a fatty liver and no pancreatic tissue. Hypophysectomy: There was no gross pituitary tissue at autopsy and sections of the thyroid and adrenal glands showed the characteristic atrophy. Houssay animals fulfilled the criteria for complete hypophysectomy and had the usual reduction in the amount of glucose excreted after pancreatectomy.

The arylsulfonylureas used were Compound U-2043 (tolbutamide; Orinase[R]) and BZ-55 (carbutamide), which were kindly provided respectively by the Upjohn Company and Eli Lilly and Company. U-2043 was in the form of a soluble sodium salt which was mixed directly with food or given parenterally in saline solution. BZ-55 was given in suspension in saline and a small amount of Tween 80. In control experiments the medium was given without the drug.

RESULTS

Table 1 summarizes the results of feeding sulfonylureas to alloxan diabetic rats. The amount of food eaten was constant for all control and treatment periods which are compared. Some control periods preceded and some followed the treatment periods, a procedure which emphasized the stability of the diabetes in each rat. On a constant diet, the proportion of the available glucose which was excreted differed in different animals. Thus, on the 15 gm. diet, rat 13 excreted 3 to 3.5 gm. of glucose per day whereas rat 16 excreted 5 to 6 gm. daily. As used in these experiments, the sulfonylureas had no effect regardless of these variations in the severity of the diabetes. Since the rat with milder diabetes (No. 13) showed no significant diminution in glycosuria and since this animal probably produced some insulin, it seems that the mere presence of insulin is not enough to insure the effect of the drugs as administered.

In the last line of table 1, the average glycosuria for all experiments means that for a total of 64 control days and 111 treatment days in four diabetic rats, there was no change in the amount of glucose excreted. The changes in weight appear to be the random variations of animals which maintained an essentially constant weight during the observations.

In addition to the above effort at treatment, a few observations were made on the response of the blood sugar to single intraperitoneal doses of 20 mg. of U-2043 per rat. In three normal rats so treated the blood sugar fell 12, 18 and 36 mg. per 100 ml. (19, 37 and 51 per cent of the initial levels). This agrees with a report from the Upjohn Laboratories (unpublished) that in twenty normal rats, given 20 to 160 mg. of U-2043, the blood sugar fell from 13 to 42 per cent of the initial level. Four diabetic rats were tested. In three there was no reduction of hyperglycemia. In the fourth, the initial blood sugar was very high, but from two to eight hours the blood sugar was constant in contrast to the decline at two to six hours in normal animals. This agrees with Achelis and Hardebeck[2] who state that alloxan diabetic rabbits do not respond uniformly and that BZ-55 appears to be without effect and might even aggravate severe alloxan diabetes. Our conclusion is that the alloxan diabetic rats did not respond to the single dose, or to the feeding, of sulfonylureas.

Table 2 shows the responses of normal cats to intraperitoneal saline and to varying doses of U-2043. The cat is clearly one of the species which responds to this drug. Cat 17 illustrates the occasional absence of the usual fall in blood sugar, an irregularity which has been encountered by other investigators.

Table 3 records the lack of effect of U-2043 on the blood sugar level of depancreatized cats. The first five animals were given only saline as controls for the treated series. When the irregularities of these blood glucose figures are surveyed it seems clear that there is no significant effect of a single dose of U-2043 on the blood glucose under these conditions. In addition to the responses to a single dose (table 3) two depancreatized cats were treated with U-2043 (100 mg. per kg. intraperitoneally twice daily) and no insulin. Both died in diabetic acidosis in three to four days after having the same amount of glycosuria as control animals. Three depancreatized cats have been treated with insulin and U-2043. No potentiation of the action of insulin could be observed in terms of the daily glycosuria on a constant diet, but the severe and labile diabetes of this species would obscure any small effect. Better results have been obtained in the dog by Sirek and Sirek.[6]

It is now recognized that pancreatectomy means not only the removal of insulin but the reaction of the pituitary:adrenal system to the loss of insulin. This is accompanied by variable degrees of insulin resistance, the exact nature of which is not well understood. Such insulin resistance might well inhibit or antagonize a drug with a weak insulin-like action; it might also obscure a weakly hypoglycemic action of some other kind. For this reason the sulfonylureas were tested in Houssay animals. Houssay cats lack insulin but, unlike simply depancreatized cats, are extremely sensitive to insulin, so that 0.25 to 0.5 unit may cause a hypoglycemic reaction. The results of the tests in Houssay cats are presented in table 4 in which eight tests in four

TABLE 1

Alloxan diabetic rats fed hypoglycemic sulfonamides

Rat number	Food per day gm.	Urinary glucose[1] Control	Treatment	U-2043 per day mg.	Change in weight Control gm.	Treatment gm.
		gm. per day				
5	20	3.9 (5)	4.5 (4)	10[2]	0	0
5	20	6.2 (8)	6.1 (9)	20[2]	+10	+10
5	20	6.2 (8)	6.6 (10)	20	—	0
5	20	6.2 (8)	6.4 (10)	40	—	+5
13	15	3.4 (7)	3.1 (13)	20	0	+5
13	15	3.5 (9)	3.1 (9)	40	+10	+10
16	15	6.2 (8)	5.2 (13)	20	−10	−10
16	20	6.6 (9)	7.0 (9)	20	+10	+10
16	20	6.6 (9)	7.2 (10)	40	—	+5
25	20	6.4 (8)	5.0 (14)	20	+10	0
25	20	4.7 (10)	5.3 (10)	40	+10	0
Averages		5.4±0.4	5.4±0.4			

[1] Days of periods are given in parentheses.
[2] These animals received BZ-55 instead of U-2043.

TABLE 2

Responses of normal cats to U-2043

Cat number	Anesthesia	U-2043 per kg. mg.	Administered[1]	Blood glucose at hours: 0	2	4	6	8	Fall in blood sugar
				mg. per 100 ml.					
15	Yes	0	IP	96	101	110	115	95	0
34	No	0	IP	72	76	74	79	77	0
36	No	0	IP	69	63	64	69	75	6
36	No	10	IP	73	56	67	67	—	17
34	No	20	IP	110	65	50	70	—	60
13	Yes	100	IP	72	41	40	27	43	45
15	Yes	100	IP	78	22	20	20	34	58
16	Yes	100	IP	84	21	31	27	27	63
17	Yes	100	IP	105	83	115	106	113	22
18	Yes	100	IP	67	34	40	27	30	40
25	Yes	100	S	105	52	62	137	107	53
26	Yes	100	S	109	79	72	95	97	37
Average of treated cats				89	50	55	64	64	44

[1] IP = intraperitoneally; S = by stomach tube.

TABLE 3

Effect of sulfonylureas in depancreatized cats

Cat number	Days after depancreatizing	Anesthesia	U-2043 per kg. mg.	Blood glucose at hours: 0	2	4	6	8	Maximum decrease
				mg. per 100 ml.					
31	1	Yes	0	289	243	249	252	282	46
38	2	Yes	0	262	233	222	193		69
34	2	No	0	277	241	247	246		36
53	2	No	0	295	305	315	289		6
53	3	No	0	270	243	276	279		27
	Averages			279	253	262	252		37
19	2	Yes	100	209	232	251	264	253	0
19	3(a)	Yes	100	301	197	250	260	279	104
20	2	Yes	100	302	242	248	241	252	60
24	3	Yes	100	315	287	288	257	248	67
8	3	Yes	100	228	225	218	202	187	41
	Averages			271	237	251	245	244	54

(a) Fourteen days after operation but three days after last insulin.

TABLE 4

Effect of sulfonylureas in Houssay cats

Cat. number	Days after: Hypox.	Days after: Depan.	Drug A=U-2043 B=BZ-55 mg. per kg.	Blood glucose at hours 0	2	4	6
				mg. per 100 ml.			
12	24	10	A–100	162	125	77	–
33	13	4	A–10	212	220	202	203
33	15	6	A–20	246	246	226	194
33[1]	20	11	A–20	319	322	315	302
33[1]	21	12	A–50	380	350	365	360
45	42	4	B–50	36	36	34	27
47	30	3	B–50	208	131	77	51
47	36	9	B–50	236	197	128	89
	Averages			225	203	178	175
29	20	5	None	38	36	27	–
33	12	3	"	206	186	154	134
33	14	5	"	224	230	217	202
33[1]	19	10	"	182	197	176	175
47	33	6	"	223	221	182	165
47	40	13	"	130	88	43	44
47	42	15	"	158	98	62	61
	Averages			166	151	123	130

[1] This animal was receiving 10 mg. of cortisone daily at the time of this test.

animals are compared to seven control curves in three animals. The average curve of each group does not show maximum fall since this occurred at different time intervals. The mean maximum fall in the treated series was 61 compared to 50 mg. per 100 ml. in the control series, an insignificant difference. It is clear that there is no effect of these sulfonylureas on the blood sugar in Houssay animals. Hypoglycemogenic compounds fail to act in the absence of insulin even in test animals that are extremely sensitive to insulin. This is strong evidence for the concept that these drugs have, of themselves, "none" of the action of insulin.

The administration of sulfonylureas in doses of 50 or 100 mg. per kg. intraperitoneally had no effect on the daily excretion of glucose by Houssay animals on a constant diet, but without insulin.

If these drugs acted by stimulating the secretion or potentiating the action of endogenous insulin, they might well be more effective in hypophysectomized animals, in which insulin sensitivity exists in the presence of an intact pancreas. Table 5 shows the results of a number of tests in hypophysectomized cats. The control tests show the occasional occurrence of slight spontaneous hypoglycemia, which is expected after this operation. The treated series shows an irregular response to the drug which is, however, quite significant on the average (mean decrease in mg. per 100 ml.=24-7=17; t=3.6). When one recalls that the initial blood sugar level is about 20 mg. per 100 ml. less than it is in normal animals (table 2), it is probable that the hypophysectomized cat has an essentially normal response to these drugs. There was no suggestion of increased sensitivity to hypoglycemia. From many tests on hypophysectomized cats in the past it is safe to say that if these drugs increased the secretion of insulin, the amount of insulin so added to the animals was certainly less than one unit, the amount which consistently causes a severe hypoglycemic reaction in hypophysectomized cats.

These results in hypophysectomized cats are unexpected in view of the report by Levine[7] of a greatly enhanced effect of BZ-55 after adrenalectomy in the rat. In a single adrenalectomized cat, tested with 50 mg. U-2043 per kg., repeated hypoglycemia and death occurred in spite of the administration of glucose. Hypophysectomy and adrenalectomy would be described as fairly similar in their effect on insulin sensitivity. However, in their effect on the response to sulfonylureas these operations are utterly different. This might well provide the background for future study.

DISCUSSION

The hypoglycemic response of normal animals to two sulfonylureas has been confirmed in the cat. The failure of depancreatized cats and of alloxan diabetic rats to react to the drugs confirms other reports. The results following pancreatectomy show that sulfonylureas do not act in the absence of insulin. This has been emphasized by the lack of action in Houssay animals which are very sensitive to insulin. The failure of alloxan diabetic rats to respond and the absence of

TABLE 5

Effect of sulfonylureas in hypophysectomized cats

Cat number	Days after hypox.	Drug[1] A=U-2043 B=BZ-55 mg. per kg.	Blood glucose at hours mg. per 100 ml. 0	2	4	6	8	Maximum decrease
42	14	0	67	75	65	75	75	2
42	21	0	67	61	67	70	70	6
45	11	0	82	90	90	85		0
45	24	0	63	65	56	50		13
47	8	0	67	67	71	67		0
47	14	0	46	41	34	45		12
49	9	0	62	58	62	63		4
50	7	0	69	69	70	63		6
50	12	0	75	84	70	45		30
51	6	0	66	65	61	68		5
51	11	0	84	82	89	85		2
54	5	0	77	92	69	78		8
	Averages		69	70	67	66		7
42	11	A–50	95	47	57	60	63	48
42	16	A–50	62	51	36	51	70	26
45	10	A–50	56	23	19	18	19	37
45	11	A–50	39	39	45	58	54	0
45	23	A–50	67	38	31	44	42	36
47	12	A–50	55	77	66	59		0
49	7	A–20	79	62	54	57		25
49	10	A–100	65	36	32	32		33
50	8	A–100	59	35	32	80		17
50	5	B–50	77	72	71	66		11
51	4	B–50	77	59	57	64		20
51	7	B–100	67	36	51	52		31
	Averages		67	48	46	53		24

[1] Intraperitoneal administration: no anesthesia.

an increased sensitivity of hypophysectomized cats to the hypoglycemic action of these drugs are noteworthy. These results mean that the islands, which almost certainly produce some insulin in both types of animal, do not measurably increase their output of insulin when sulfonylureas are given. It is perhaps possible that the tendency to lowered blood sugar levels counteracts or offsets this stimulation although it does not do so in normal animals. In any case, no appreciable secretion of insulin occurs in the diabetic rat which needs it or in the hypophysectomized cat which ought to reveal the presence of extra insulin because of its characteristic sensitivity.

If these drugs had no effect on the secretion of insulin but enhanced or potentiated the insulin in the blood or tissues, one or both of these test animals ought to show it. These results, as well as clinical studies in which the patient's dose of insulin sometimes can and sometimes cannot be reduced, suggest that something more than the presence of insulin is needed to provide the conditions in which the drugs act. This thought is further supported by the unexpected difference between the response of hypophysectomized and that of adrenalectomized animals. De Bodo and Sinkoff[11] describe "the greater insulin sensitivity of the hypophysectomized dog . . . compared with that of the adrenalectomized dog." If this applies to other species, adrenalectomized cats or rats are not more sensitive to insulin than their hypophysectomized counterparts, but in our experiments differ directly in their response to sulfonylureas. Such a difference might aid in the elucidation of the action of these drugs in the future. At present one cannot tell whether the difference between hypophysectomized and adrenalectomized animals resides in the liver, the islands or other tissues. The evidence that sulfonylureas do not appreciably alter insulin secretion must in the long run be viewed with results such as those of Ashworth and Haist[6] who reported growth of the islands during prolonged treatment of rats with BZ-55.

SUMMARY

The effect of BZ-55 (carbutamide) and U-2043 (Orinase^R) has been observed on the blood sugar level and on experimental diabetes of certain types. Normal cats responded to these drugs with a fall in the blood sugar level. There was no demonstrable effect on the diabetes of alloxan diabetic rats or depancreatized cats untreated with insulin. There was no apparent effect

on the diabetes of three depancreatized cats treated with insulin, but the cat is not recommended for this type of study. The drugs failed to exert an effect on the blood glucose or daily glycosuria of hypophysectomized-depancreatized cats in spite of their known sensitivity to insulin. Hypophysectomized cats have an approximately normal response of the blood sugar in contrast to the great sensitivity of adrenalectomized animals to these drugs. The experiments indicate that these sulfonylureas have little or no effect on the secretion or potentiation of insulin unless this be the mechanism of its action in the adrenalectomized animal.

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Effect of Carbutamide on the Insulin Content of the Dog Pancreas

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One of the mechanisms of action postulated for the hypoglycemic effects of the group of arylsulfonylurea compounds of which carbutamide† is a member, is a stimulation of the β-cells of the pancreas to secrete, or produce and secrete, more insulin. If this is the manner in which such compounds lower blood glucose levels, two possibilities exist as to the final result of such therapy. The islets of Langerhans may be stimulated to produce more or larger β-cells or the existing cells may be stimulated to secrete more insulin per unit of time. If this enhancement of insulin production can be maintained throughout many years, the stimulation would be beneficial to the mild diabetic. If the stimulation causes oversecretion of the cells and consequent exhaustion, however, a patient with mild diabetes might conceivably become a severe diabetic, a far from desirable effect.

In order to discover the effect of carbutamide on the insulin content of the pancreas, dogs were treated with the drug for various periods of time, killed, the pancreas extracted and the extract assayed for insulin content.

METHODS

Dogs, caged separately, were maintained in air-conditioned quarters and allowed water ad libitum. In experiment 1 the animals were fed Friskies dog food ad libitum and the daily consumption was measured. In experiments 2 and 3 the dogs were regulated on a single daily feeding of Pard dog food in an amount that would be eaten within the twenty-four-hour period (usually within one hour of feeding) and would produce a slow weight gain.

The dogs were weighed every other day. Placebo capsules containing acacia were given to control dogs in experiment 1 but not in experiments 2 and 3. Carbutamide was administered twice daily by mouth. The doses varied in the different experiments. Blood glucose

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† Carbutamide, p-aminophenylsulfonyl butylcarbamide, is also known as BZ-55, and in Europe, by the trade names Nadisan and Invenol.

and carbutamide concentrations were measured once each week before and after drug administration. In experiment 1 blood samples were taken at 8 a.m. (before the morning dose of drug), 12 m. and 4 p.m. (before the afternoon dose of drug). In experiment 2 the blood samples were taken at 8 a.m., 10 a.m. and 4 p.m. and in experiment 3 at 8 a.m. (pre-drug) and 10 a.m. (two hours post-drug) when the effect of the morning dose should be near its peak. Blood from ear vein was used throughout. The Hagedorn-Jensen[1] method was used for glucose and the Bratton and Marshall[2] method for carbutamide.

The dogs were killed with intravenous sodium secobarbital about eighteen hours after the last dose of drug. The pancreas was removed, weighed, a small piece fixed in Bouin's solution for histological examination, and the remaining portion frozen quickly between slabs of dry ice. Pieces of liver, heart and skeletal muscle were frozen for determination of glycogen content. The following organs were examined grossly and microscopically for pathological changes: liver, kidney, lung, heart, adrenal, thyroid, spleen, and small intestine. This examination is not complete and will be reported later.

Each frozen pancreas was cut into small pieces and ground for one minute in a Waring Blendor containing an acid-alcohol solution. After filtration the residue was again ground with acid-alcohol and filtered. Four volumes of absolute alcohol were added to the combined filtrates. Sixteen volumes of ether were then added to the filtrate and insulin was allowed to precipitate out in the cold overnight. The precipitate was then collected in a small filter. The insulin was dissolved in a small amount of insulin diluting fluid (U.S.P.). These insulin solutions were then further diluted with insulin diluting fluid and assayed in the mouse convulsion test against a standard insulin preparation.

The glycogen of liver, muscle and heart was determined by standard methods.[1, 3] Glycogen concentration is expressed as milligrams glucose per gram wet weight of tissue.

RESULTS

There were eleven dogs in experiment 1. Of these, three were controls (group A); three received carbutamide orally in a priming dose of 2 gm. followed by 1.2 gm. twice daily (group B); three received 2 units of NPH insulin subcutaneously twice daily (group C); and two received both carbutamide and NPH insulin twice each day (group D). These dogs were killed after fourteen days of treatment. In table 1 are recorded the mean values of body weight, blood glucose and blood carbutamide concentration of each group for each week. All dogs except one lost weight during the experimental period and those in the control group lost almost as much as did the two groups treated with carbutamide. Most of the weight losses can be attributed to decreased food consumption. In all dogs receiving carbutamide the dose was decreased during the second week but the blood levels of drug remained in the toxic range until the end of the experiment.

In figure 1 are presented the mean values for insulin and glycogen concentration in the tissues of these dogs. The extractable insulin of the pancreas was greatly decreased in all of the dogs treated with carbutamide and there was no overlap between these groups and the

TABLE 1

Experiment 1. Mean values for body weight, and blood glucose and carbutamide levels for each group of dogs.

Group	Body weight kg.	Blood glucose—mg. per cent 8 a.m.	Noon	4 p.m.	Blood carbutamide—mg. per 100 ml. 8 a.m.	Noon	4 p.m.
Pre-drug							
A	7.08	86	93	91			
B	9.52	87	69	74			
C	7.43	84	81	77			
D	8.33	81	92	86			
7 days treatment							
A	6.45	84	96	91			
B	9.15	81	98	78	74	100	96
C	7.40	84	93	89			
D	8.15	64	74	60	103	108	140
14 days treatment							
A	6.17	79	84	91			
B	7.73	49	67	52	114	132	123
C	6.90	70	63	79			
D	6.70	50	51	57	94	91	85

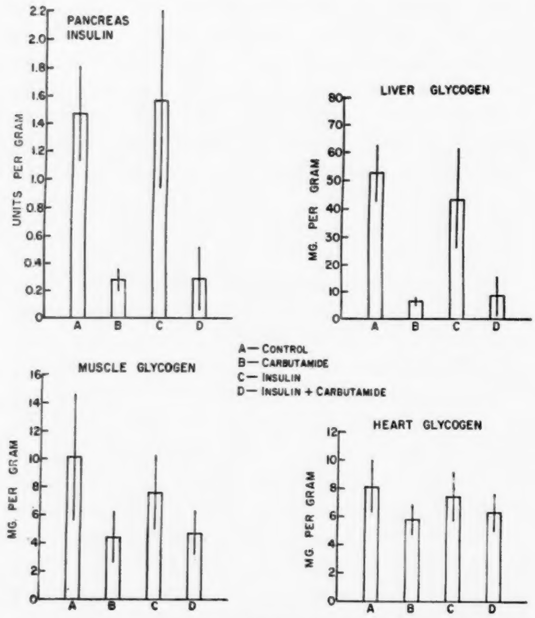

FIG. 1. Experiment 1. The effect of fourteen days' treatment with carbutamide on extractable insulin of the pancreas and on tissue glycogen concentration in dogs. The columns represent the mean value for each group and the vertical line through each column indicates the standard error of the mean.

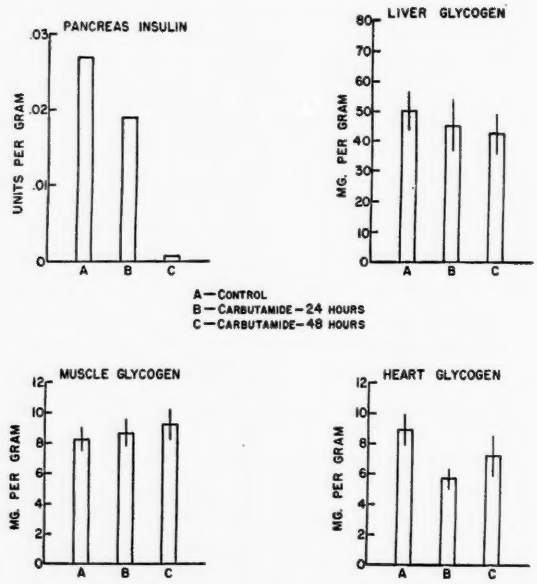

FIG. 2 Experiment 2. The effect of twenty-four to forty-eight hours' treatment with carbutamide on the extractable insulin of the pancreas and on tissue glycogen concentration in dogs. The columns represent the mean value for each group and the vertical line through each column indicates the standard error of the mean. The S.E. was not calculated for the insulin values since the accuracy of these is questionable.

controls or those treated with insulin alone. The highest insulin concentration in the carbutamide group was 0.58 units per gm., whereas the lowest value for the control groups was 0.95 units per gm. If one combines groups A and C and compares them with combined groups B and D the difference between the two means has a P value of less than 0.01.

The decreases in tissue glycogen found in the carbutamide-treated animals are more difficult to evaluate. The decreased food consumption and consequent undernutrition might possibly be responsible although the degree of weight loss in the four groups did not vary greatly. With so few animals in each group these results can only suggest possibilities that need further study.

In the second experiment eleven dogs were divided into three groups. Group A (five dogs) was a control, group B (three dogs) received carbutamide for twenty-four hours, and group C (three dogs) received carbutamide for forty-eight hours. The doses of carbutamide were smaller than in experiment 1 and blood levels ranged from 5 to 24 mg. per 100 ml. with a mean of 12. The mean blood glucose before treatment was 78 mg. per cent and fell to 65 mg. per cent two hours after the first dose of carbutamide. The dogs treated for twenty-four hours received a total dose of 2 gm. each and those treated for forty-eight hours received 3 gm. each.

The values for insulin concentration of these pancreases were very low (figure 2). A modified extraction procedure was used which apparently removed only a portion of the insulin. If it is assumed that the proportion of insulin extracted from each pancreas was the same, these values indicate that the insulin content falls within twenty-four to forty-eight hours after the start of drug administration. In this experiment there were no significant changes in muscle glycogen and the decreases in heart glycogen were not consistent enough to be evaluated on the basis of so few animals. The reductions in liver glycogen were too small to be significant when so few animals are used, but there seemed to be a trend toward lower glycogen values as drug therapy was continued.

The third experiment included sixteen dogs of which four were controls and received no drug (group A). Groups B (six dogs), C (three dogs) and D (three dogs) received carbutamide daily for eight weeks in

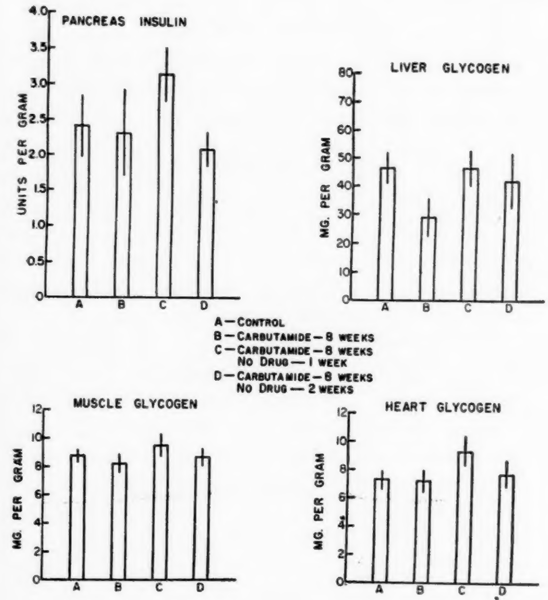

FIG. 3. Experiment 3. The effect of eight weeks' treatment with carbutamide on the extractable insulin of the pancreas and on the tissue glycogen concentration in dogs. The columns represent the mean value for each group and the vertical line through each column indicates the standard error of the mean.

doses sufficient to maintain blood levels below the toxic range. At the end of eight weeks, group B was killed and drug administration was stopped on groups C and D. Group C was killed one week and group D two weeks after halting the drug. All the dogs in this experiment gained weight and none showed symptoms of drug toxicity. The blood level of carbutamide in the morning prior to drug administration ranged from 4 to 19 mg. per 100 ml. with a mean of 7.7 and two hours after carbutamide the range was 9 to 29 mg. per 100 ml. with a mean of 16.0. The doses of carbutamide were sufficient to decrease blood glucose in all dogs.

In figure 3 are shown the insulin and glycogen concentrations in the tissues. The extractable insulin of the pancreas was normal in all the treated animals and there were no differences in heart or muscle glycogen concentration in the four groups. However, again there was a suggestion that carbutamide therapy decreased liver glycogen concentration but that the glycogen returned to normal levels within a week after stopping the drug. Here again the number of animals in each group was too small for the changes to be statistically significant.

DISCUSSION

In each of the three experiments reported, the number of dogs was small and the changes in insulin extractable from the pancreas and in glycogen concentration of the tissues therefore are difficult to evaluate. Although significant decreases of insulin and glycogen concentration were seen in the carbutamide-treated dogs of experiment 1, the decreased food intake, muscular weakness and lethargy produced by toxic blood levels of carbutamide may well have exerted an effect on these tissue constituents. Although the dogs in the control group also lost weight, they did not exhibit the symptoms of drug toxicity seen in the carbutamide-treated dogs.

Experiment 2 also is open to considerable criticism since it is obvious that the process for extracting insulin was inadequate and that the quantity recovered from pancreases of normal control dogs was only 2 per cent of the expected amount.

The results of the third experiment suggest that even if doses of carbutamide that produce therapeutic blood levels may decrease the insulin content of the pancreas when drug therapy is first started (experiment 2), longer treatment does not maintain this situation and the pancreas recovers its normal insulin concentration.

The decreases in liver glycogen concentration observed in all three experiments are contrary to previous reports of the effects of arylsulfonylureas on this constituent. Miller and Dulin[4] found an increase of from 140 to 240 per cent in liver glycogen concentration in rats seven hours after administration of 240 mg. per kg. of tolbutamide* orally. Tyberghein, Halsey and Williams[5] also reported an increase in liver glycogen concentration after administration of about the same dose of tolbutamide to fasted rats, but no effect if the animals had been fed glucose at the time the drug was administered. The experiments described here are of longer duration than those reported by others and the results may reflect a homeostatic adjustment that requires twelve hours or longer for attainment. At present there is not sufficient evidence to determine the mechanism of this decreased liver glycogen concentration.

SUMMARY

1. Daily administration of carbutamide (p-aminophenylsulfonyl butylcarbamide) for fourteen days to dogs in doses producing blood levels of drug in the toxic range caused a severe decrease in the amount of insulin extractable from the pancreas. Liver glycogen

* Tolbutamide, butyltolylsulfonylurea, is also known as D-860 and by the trade name Orinase.

concentration was also decreased in treated dogs as compared with untreated control animals.

2. The decrease in extractable insulin was measurable after twenty-four hours of treatment with carbutamide in doses producing blood levels within the therapeutic range, and was greater after forty-eight hours treatment.

3. Eight weeks' treatment of dogs with carbutamide in doses sufficient to maintain therapeutic, but not toxic, blood levels did not cause any lasting changes in pancreatic insulin concentration.

4. Both short-term (twenty-four to forty-eight hours') and chronic (eight weeks') treatment with carbutamide caused decreases in liver glycogen concentration which, although not statistically significant for the small numbers of animals in each group, were consistent in each of the three experiments.

ACKNOWLEDGMENTS

I am indebted to Dr. Paul N. Harris for performing complete autopsies on all the dogs and for dissection of the tissues used in these experiments.

Dr. C. A. Kuether and Mr. E. R. Diller of the Division of Biochemistry kindly prepared the pancreatic extracts and supervised the bioassays for insulin activity.

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Studies on the Mechanism of Action of Orinase (Tolbutamide)

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With the exception of a few studies employing Orinase in human volunteers our experiments have been limited to an exploration of the possible effect of the sulfonylureas on certain enzyme systems. I should like to summarize briefly and then to discuss a few of our experiments which indicate that the hypoglycemic effect of these compounds may be due to interference with glycogenolysis in the liver, thus diminishing the release of glucose into the blood.

In the course of a survey of a large number and variety of compounds for insulinase inhibitory activity, the effects of Orinase and BZ-55 were tested on a partially purified rat liver insulinase. The term insulinase is used to refer to an enzyme or group of enzymes which have proteolytic activity toward insulin. At 3×10^{-3} molar concentration no inhibition was observed with either of these compounds in an assay system containing I^{131} labeled insulin, Tris buffer and versene. Dr. Mirsky has found inhibition of insulinase activity in a similar system using concentrations of Orinase and BZ-55, 3 to 15 times this magnitude.[1]

In further studies we examined the effects of the sulfonylureas on the conversion of liver glycogen to blood sugar since it seemed that an action at this site might explain many of the early data on their in vivo effects. It was conceivable that hypoglycemia was due to an inhibition of the activity of the liver glucose-6-phosphatase. A similar situation is found in one type of glycogen storage disease where liver glycogen is maintained at abnormally high levels in the face of hypoglycemia, due to a deficiency of glucose-6-phosphatase. Glucose-6-phosphatase activity was assayed in homogenates of rat and of rabbit liver according to the method of Cori and Cori.[2] In these experiments we were able to demonstrate no effect of Orinase at a concentration of 5×10^{-3} M, nor was it possible to obtain an effect of Orinase on the release of glucose by rat or rabbit liver slices incubated for thirty minutes in vitro. The data on glucose release by liver slices, six experiments with rat liver and nine with rabbit liver, are shown in table 1. For each experiment successive

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slices from a single piece of liver were used. It will be seen that the presence of Orinase at a concentration of 5×10^{-3} M had no effect on the glucose release. The value in parentheses is the mean of the percentage effects of Orinase calculated for each pair of slices, one with and one without the drug. The last column contains the standard errors of the respective means.

Table 2 summarizes in a similar fashion some of the data on the effect of epinephrine on glucose release by liver slices. In this case the addition of Orinase at a concentration of 5×10^{-3} M is associated with a marked decrease in the epinephrine effect. This has been repeated many times in experiments not included in this figure and similar results have been obtained. Several lower concentrations of Orinase also have been used. In a short series of experiments with 5×10^{-4} M Orinase the epinephrine effect was decreased about 40 per cent. Using a similar protocol, a few preliminary studies were done employing amorphous insulin as a source of glucagon activity. These are shown in table 3. It appears that Orinase also inhibits the glucagon effect on glucose release by liver slices. Recently a series of experiments was done using purified glucagon at a final concentration of 19 μgm. per ml. In this group of nine experiments the glucagon effect was consistently decreased in the presence of 5×10^{-3} M Orinase. The mean inhibition due to Orinase was about 90 per cent.

It seemed possible that inhibition of the increased glucose output induced by epinephrine or glucagon in these experiments could be due to an inhibition of either phosphoglucomutase or of glucose-6-phosphatase (although the latter could not be demonstrated directly), which would not be sufficient to alter the control rate of glucose ouput but would be enough to make one of these reactions the rate limiting step in glucose release when the phosphorylase step was accelerated. An attempt was made to explore this possibility by determining the effect of Orinase on the glucose output when glucose-1-phosphate was added to the medium. The results of these experiments are shown in figure 1. The first three bars show the glucose output of the controls, the slices with epinephrine alone and epinephrine plus Orinase. The last three bars show the glucose output with glucose-1-phosphate alone, plus epinephrine and plus epinephrine and Orinase. (The data are not included here but Orinase had no effect on the output of glucose in the presence of glucose-1-phosphate alone.) It is apparent that even in the presence of Orinase the addition of glucose-1-phosphate leads to a great increase in glucose output (last bar) and that the small

TABLE 1

Effect of Orinase on glucose release by liver slices

	Orinase 5×10^{-3} M.	mg. glucose per gm. liver Mean	SEM
Rat (6)	0	6.1	0.60
	+	6.1	0.45
(Orinase effect)		(+2.3 per cent)	(8.8)
Rabbit (9)	0	4.7	6.48
	+	4.3	0.41
(Orinase effect)		(−7.0 per cent)	(5.6)

TABLE 2

Effect of Orinase on glucose release by liver slices incubated with epinephrine

	Orinase 5×10^{-3} M.	Epinephrine effect* Mean	SEM
Rat (5)	0	+3.0	0.44
	+	+1.1	0.27
(Orinase effect)		(−61.9 per cent)	(10.7)
Rabbit (10)	0	+3.2	0.26
	+	+0.6	0.22
(Orinase effect)		(−85.7 per cent)	(7.6)

* Extra mg. glucose released per gm. of liver due to epinephrine.

TABLE 3

Effect of Orinase on glucose release by rabbit liver slices incubated with "glucagon" (amorphous insulin)

Experiment	"Glucagon" effect mg. glucose per gm. liver Control	Orinase	per cent effect
1	+2.6	+1.2	−54
2	+3.3	+1.7	−49
3	+1.6	+0.7	−47
4	+3.2	+1.2	−63
Mean	+2.7	+1.2	−53

inhibition noted when both epinephrine and glucose-1-phosphate are present corresponds roughly to the increment in glucose output associated with the addition of epinephrine.

All of our data are consistent with the view that these hypoglycemic agents interfere with the conversion of liver glycogen to glucose. Although alternative explanations have not been excluded, it is suggested that they may act by inhibiting the phosphokinase which catalyzes the formation of active phosphorylase so that it is less susceptible to activation by epinephrine and

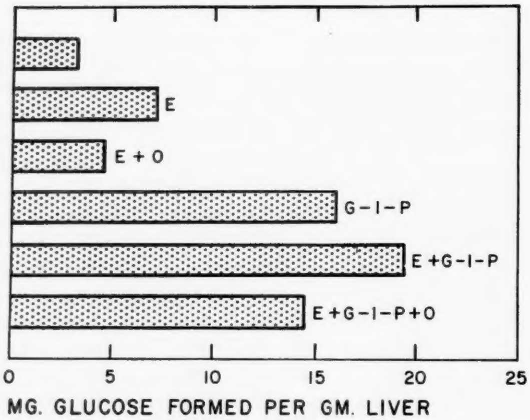

FIG. 1. Glucose release by rabbit liver slices with additions as noted on each bar. E = epinephrine, O = Orinase, G-I-P = Glucose-I-phosphate.

glucagon. An action at this site would also explain most of the observations obtained during the use of these compounds in vivo. It now seems likely that BZ-55 and Orinase do not cause damage to the α-cells of the pancreas. Further, there is no direct proof that these drugs possess an insulin-like action or are able to increase the amount of circulating insulin or potentiate its action. In fact, there are several reports of effects of the sulfonylureas which are unlike those of insulin. Finally, I should like to mention just a few of the published experiments which are indicative of an effect of these compounds on the conversion of liver glycogen to glucose.

Dulin and Miller[3] found that liver glycogen levels in fasting rats given a single dose of Orinase were higher than those of the control groups. Baender and Scholz[4] have recently reported that doses of Orinase which are within the therapeutic range will lead to elevated liver glycogen levels in rats and guinea pigs. In addition, it has been found by Creutzfeldt and Finter[5] that the hyperglycemic response to a test dose of glucagon is decreased, although not abolished, in rabbits receiving Orinase. In these studies it was determined that the liver glycogen levels of the test animals were within the normal range so that the diminished response to glucagon presumably was not due to a deficiency of glycogen. Anderson[6] has measured blood sugar levels in the hepatic vein and in the femoral artery of the dog during the postabsorptive period and has found that there are constant, irregular fluctuations in the glucose concentration at both of these sites. These fluctuations are abolished by the administration of Orinase which at the same time causes a marked decrease in the rate of glucose output by the liver. He has found that these Orinase effects can be completely reversed by glucagon given intravenously. In addition, several workers have reported that it is possible to obtain a hyperglycemic response to epinephrine injection in patients receiving Orinase. In general, rather large amounts of epinephrine have been used and it is difficult to compare quantitatively the epinephrine response with and without the drug. We attempted to do this in a few normal humans and were unable to interpret the data due to daily variations in the response to either drug alone. However, in a small number of in vitro experiments we have obtained results which, if confirmed, may bear on this question. In recent studies, it appears that the Orinase inhibition of the epinephrine effect on glucose release by liver slices may be at least partially overcome by increasing the concentration of epinephrine. If the relative concentrations of Orinase, epinephrine and/or glucagon are important among the factors determining the blood sugar level in a given case most of the in vivo observations, some of them apparently contradictory, may be reconciled and explained in the framework of the hypothesis based on in vitro studies.

For the sake of brevity I have omitted many details concerning our experiments, and have presented an obviously very limited discussion of one interpretation of the data.

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Group Discussion

JOSEPH IZZO, M.D., (*Rochester, New York*): Do I understand, Dr. Mary Root, that you have found no effect of carbutamide on liver glycogen?

MARY ROOT, PH.D., (*Indianapolis*): Our experiments are long-term ones, and if there is any effect at all, it is a decrease in liver glycogen after drug administration for one day to eight weeks. The liver is taken eighteen hours after the last dose. This is a different type of experiment from those previously reported to show increases in liver glycogen.

STEFAN S. FAJANS, M.D., (*Ann Arbor*): In relation to Dr. Lukens' paper, at the March conference we reported on a patient with diabetes mellitus and concomitant partial panhypopituitarism. A thirty-year-old male with craniopharyngioma failed to grow and also showed evidence of complete hypothyroidism and partial adrenal insufficiency. This individual is extremely sensitive to insulin. In this case BZ-55 had no consistent effect on the blood sugar, an observation in agreement with the experiment on the Houssay animal.

Again in correlation with Lukens' failure to find increased sensitivity to exogenous insulin, we could demonstrate none in our case following large amounts of BZ-55, although blood levels were 20 mg. per 100 ml. or more.

In relation to Dr. Vaughan's studies, both in normal and diabetic patients who respond to the sulfonylureas, we found no differences in the hyperglycemic effect to adrenalin and glucagon whether these substances were given by themselves or with BZ-55 and Orinase.

ROBERT H. WILLIAMS, M.D., (*Seattle*): With reference to Dr. Lukens' discussion, we have carried out studies with hypophysectomized and adrenalectomized rats. As in his studies with cats, we found that there was a normal hypoglycemic response to carbutamide in the hypophysectomized animal whereas there was a markedly accelerated response in the adrenalectomized rats.

M. E. KRAHL, PH.D., (*Chicago*): I would like to comment briefly on Dr. Lukens' and Dr. Vaughan's papers. These seem to me to emphasize the problem of the mechanism of action of these drugs.

If these observations of Lukens on the hypophysectomized cat are substantiated as they seem to be from Dr. Williams' comments, an explanation of a carbutamide effect based on either inhibition of insulinase or discharge of insulin from the pancreas would be ruled out.

In commenting on Dr. Vaughan's paper, and in general on effects on liver output of glucose produced by carbutamide, I ask the question: If the effect in the normal animal is due to an inhibition of glucose output, why is this not seen in a diabetic animal as well?

LAURANCE W. KINSELL, M.D., (*Oakland*): In relation to Dr. Lukens' paper, we have until now refrained from using carbutamide or tolbutamide in our totally hypophysectomized diabetics because of the precarious state of their vasculature and the feeling that we were not justified until the toxicity picture became clearer. We have, however, used carbutamide in one patient who is less than totally hypophysectomized but who, nonetheless, has evidence of some pituitary insufficiency. This man had received carbutamide before and after hypophysectomy. In general, he had some modification of insulin requirement on both occasions, but there was no significant difference posthypophysectomy as compared to prehypophysectomy. This also would confirm Dr. Lukens' observations.

R. E. HAIST, M.D., (*Toronto*): I should like to ask Dr. Lukens and Dr. Krahl if they think the absence of an effect in the hypophysectomized animal would preclude an influence on the pancreas mediated through pituitary or some other gland.

DR. KRAHL: It may not be a direct effect.

DR. MARY ROOT: In relation to Dr. Lukens' and Dr. Williams' results, Dr. Houssay reported at the Physiological Congress this summer that he also had found that there was no increase in insulin sensitivity in the hypophysectomized animal whereas there was an increase in the adrenalectomized animal. Houssay stated that you can save the adrenalectomized animal by giving tremendous amounts of glucose. But he asked: "Where is this glucose going?"

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): Dr. Fajans and Dr. Kinsell point out that man appears to behave like our experimental animal; Dr. Williams has given confirmation of this. The story I presented was a preliminary one, but perhaps might stand up as a physiological finding.

Dr. Krahl asks: "Why, if these drugs inhibit the discharge of glucose from the liver in the normal, don't they do so in the diabetic?" Two reasons occur to me on purely theoretical grounds: These drugs will act on a liver only when it is conditioned to a certain rate of metabolism. One might use that same argument, in the hypophysectomized animal for example. The liver, op-

erating at a minus 30 BMR, is so depressed it cannot respond further.

GEORGE ANDERSON, M.D., (*Brooklyn, New York*): A great difference in the calculation of the liver glucose output would depend on where the blood sample is taken. If you take it from the periphery it would not reflect change in the output of glucose by the liver. If it is taken directly from the hepatic vein the estimation would be more valid.

We have found that if one studies the hepatic vein flow directly, one can determine a decreased output by comparing that with the vena caval blood. In other words, if there is the same output into the hepatic vein as occurred before sulfonylureas were given, that should be preliminarily higher because it represents the arterial side. We have been finding that there is a decreased output into the hepatic vein even in the diabetic animal, even though this cannot be detected by the ordinary blood sugar methodology.

DR. LUKENS: I agree with Dr. Anderson that it's very important to measure it that way.

DR. IZZO: With reference to Dr. Krahl's question on glucose output: Does anybody have any information on the glucose output and the effect of this on liver slices in diabetic animals?

UNIDENTIFIED: There is no difference in glucose production.

DR. WILLIAMS: I want to comment on the inhibition of the degradation of insulin by these drugs when used in the usual therapeutic dosage. There is no question, as Dr. Vaughan indicated, that if one uses large quantities of these sulfonylureas in vitro there is marked inhibition of the degradation of insulin. Furthermore, this fact can be demonstrated by incubating the liver enzyme preparation with insulin with these inhibitors, and then testing various mixtures in rats, noting the degree of lowering of the blood sugar. There is no doubt that these sulfonylureas, when incubated in vitro with insulinase, will spare the degradation of the insulin.

We carried out two further types of experiment. The sulfonylureas were first given to the animal by gavage, in doses of 300 or 500 mg. per kg. These doses were definitely hypoglycemic. After a period of two hours, the liver was removed; on incubation in vitro no inhibition of the degradation of insulin was demonstrated. If all of the compound that we gave were in the liver, it still would not inhibit the degradation of insulin if one judges from the concentration that was found necessary in our in vitro studies.

In another experiment, we gave labeled insulin intraperitoneally to baby rats, and simultaneously gave the sulfonylureas by gavage or intravenously. We could not demonstrate any inhibition of the degradation of I^{131}-insulin. These conclusions are not in accord with those of Dr. Mirsky. There were some differences in technic but I would be inclined to conclude that, in the doses that are usually used therapeutically in diabetics, inhibition of degradation of insulin does not seem to be significant.

EARL W. SUTHERLAND, JR., M.D., (*Cleveland*): I'd like to ask Dr. Mary Root about the glycogen values because this bears on the mechanism of action. This is one place where the action of these compounds differs from the action of insulin. I wonder if it isn't because of the length of time after drug administration. As I recall, you reported some time previously the effect of glucagon on prolonged administration and found some hours after giving glucagon actually an increase in glycogen. I wonder if that result, and this finding of decreased glycogen may have something to do with the time period of sampling. I understood that you sampled as long as eighteen hours after the last dose.

DR. MARY ROOT: Yes, I sampled approximately eighteen hours after the last dose. Certainly there was a difference in the experiment in which we gave the animals insulin. The liver glycogen values were about the same as those in the controls, whereas the ones that had had carbutamide alone or carbutamide plus insulin had low liver glycogen values. These findings probably fit in with the time factor. It's going to take an experimental animal such as a rat to do this because the actual variation from animal to animal in the liver glycogen concentrations is so great in dogs, that since we only run three or four we don't really get statistically significant results on account of the small changes that occur.

DR. SUTHERLAND: One other comment regarding the liver slice and glucose output perhaps should be saved till later, but we find different results depending on the dose level. We do need to go to fairly high dose levels before we see these effects in liver slices—much like Dr. Vaughan. When concentrations are at a level around 1×10^{-3}, close to the therapeutic range, we see very little effect on glucose output by the liver slice.

MARTIN G. GOLDNER, M.D., (*New York*): In connection with Dr. Williams' comments on insulin degradation, Dr. Sol Berson in our laboratory from his studies is convinced that in therapeutic doses of the sulfonylureas there is no evidence of inhibition of insulin degradation. Neither is there, as Dr. Berson assures me, any inhibition of glucagon inhibition.

Some Effects of BZ-55 (Carbutamide) in Experimental Animals

R. E. Haist, M.D., Rosemary D. Hawkins, Ph.D.,† and M. A. Ashworth, M.D.,‡ Toronto*

One of the most outstanding effects of carbutamide is its effect in reducing the level of sugar in the blood. This might result from some change in the removal of glucose from the blood or from some change in the entry of glucose into the blood, under the influence of certain hormones, especially insulin. There is little evidence to support a hypoglycemic effect of carbutamide resulting from increased glucose removal from the blood. There is some evidence for an effect of carbutamide on mechanisms involved in the supply of glucose to the blood and there is some evidence in favor of an influence of carbutamide on insulin secretion and on the insulin-secreting structures.

The literature on these points will not be reviewed now, nor will the material presented at the last conference on BZ-55 be reviewed. We shall attempt to outline briefly some experiments which have been undertaken at the Charles H. Best Institute at Toronto since the last meeting. The results of parts of these investigations have been published in the *Canadian Medical Association Journal*.[1]

One of these effects concerns the islets of Langerhans. When rats were given BZ-55 by mouth for three to five weeks at doses of 0.5 to 1.0 gm. per kg. of body weight daily, an increase in islet weight was noted. Previously, Mr. B. Kinash found that continuous intravenous infusion of BZ-55 at the rate of approximately 1 gm. per kg. per day for one week did not lead to a significant increase in islet tissue. With the longer period of oral administration of the BZ-55, however, the effects seen in figure 1 were found. It is evident from this figure that there was an increase in islet weight in the rats treated with BZ-55. This was significant at the 1 per cent level. At the same time there was a significant decrease in pancreas weight, thus increasing the concentration of islet tissue in the pancreas. The islet weight per unit of body weight was also significantly increased.

This finding supports the view that BZ-55 somehow, directly or indirectly, stimulates the islets of Langerhans and that this stimulation persists over a period of time.[2] If this be true, then there is the possibility that, when the islet tissue has been reduced by any means (as for example by partial pancreatectomy), the administration of BZ-55 may excessively stimulate and exhaust the remaining islet cells and hence have a harmful effect. A number of rats were partially depancreatized and in no instance was glycosuria observed in the period following the partial pancreatectomy. When BZ-55 was administered orally at a dose of 0.5 gm. per kg. per day for five weeks, one rat began to show glycosuria while still on BZ-55 and the glycosuria disappeared in a few days after the BZ-55 was discontinued. These findings are hard to interpret at present. The experiment is still in progress. There are two major difficulties in this experiment. The first is that it is not possible to know the exact proportion of the pancreas removed and the second is that the rat islets are very difficult to exhaust through overwork.

Could BZ-55 influence the blood sugar level in other ways than by stimulating the islets? There are many other effects, to be sure, but the relation of these effects to the presence of insulin is not yet clear. BZ-55 may influence the time taken for glucose to leave the gut and it may affect also certain mechanisms involved in the handling of glucose in the liver.

The removal of glucose from the gut is influenced by a single injection of BZ-55 in the rat. Mr. T. L. Friedlich and others in our laboratory showed that the intravenous injection of BZ-55 (2 cc. of a 5 per cent solution of BZ-55 into the tail vein) three hours before glucose was administered by mouth led to a reduction in the rate of removal of glucose from the gut.[3] This was shown by the presence of a greater residue of reducing substances in the gastrointestinal tracts of the BZ-55-treated rats as compared to the residues in control animals given glucose but no BZ-55 and sacrificed at the same times (figure 2). When the BZ-55 was given by mouth over a period of three weeks, the effect was somewhat similar but less marked and not so consistent. The differences that did occur were in the residual reducing substances of the stomach. This would suggest that the effect is probably not an effect primarily on absorption but rather on the movement of material along the gut, hence on gastrointestinal motility. Certain

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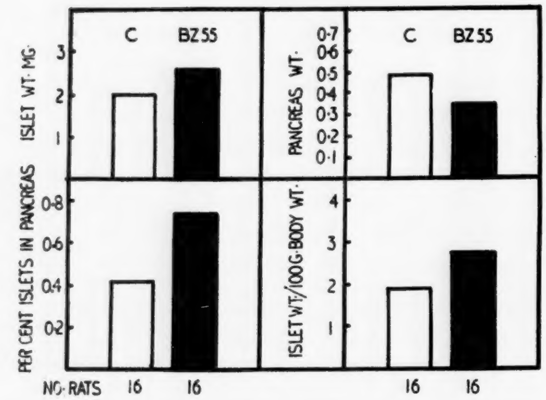

FIG. 1. The effect of administering BZ-55 for three to five weeks on the islet weights and pancreas weights in rats. C=mean values for control rats. BZ-55=mean values for BZ-55-treated rats. (From Ashworth and Haist, reprinted with permission from Canad. M.A.J. 74:975, 1956.)

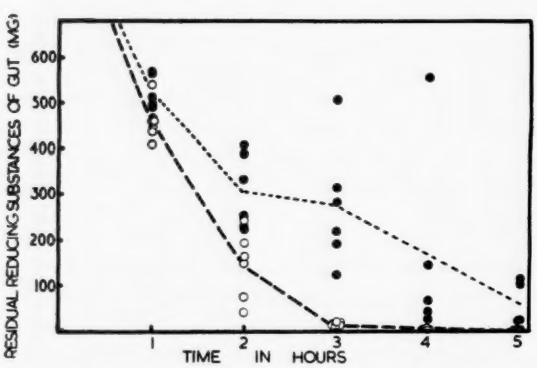

FIG. 2. The effect of BZ-55 on residual reducing substances of the gut. The solid dots represent the values for the residual reducing substances of the gut for rats receiving BZ-55 intravenously three hours before glucose was given. The open circles represent residual reducing substances of the gut for control rats. One gram of glucose was given by stomach tube at time zero. Each dot or circle represents a different rat. (From Friedlich, et al., reprinted with permission from Canad. M.A.J. 74:973, 1956.)

sulfonamides have been reported to inhibit peristalsis.[4] If BZ-55 has such an action it might explain the effect we have just outlined.

One further investigation of interest concerned the effect of BZ-55 on glucose-6-phosphatase activity. It was found that BZ-55 administration to rats for three weeks led to a reduction in the glucose-6-phosphatase activity of the liver. This will be evident in figure 3. Since it has been reported that insulin reduces the glucose-6-phosphatase activity of liver,[5] there was the possibility

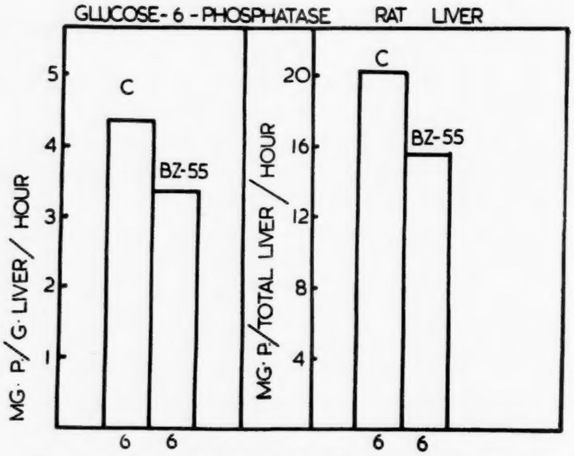

FIG. 3. The effect of the administration of BZ-55 on the glucose-6-phosphatase activity of liver. C=mean values for control rats. BZ-55=mean values for rats receiving BZ-55 in the diet for three weeks.

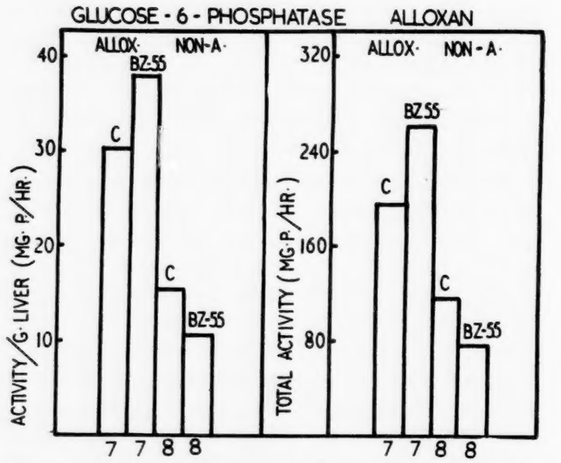

FIG. 4. The effect of the administration of BZ-55 on the glucose-6-phosphatase activity of liver in alloxanized (ALLOX.) and nonalloxanized (NON-A.) rats. C= mean values in control animals. BZ-55=mean values for rats receiving BZ-55 for eight days. In this experiment ten times the substrate concentration of the previous experiment was used because this was found to give the optimal effect.

that this effect of BZ-55 was due to insulin release. For this reason a study was made of the effect of BZ-55 on the glucose-6-phosphatase activity of the liver in rats made diabetic with alloxan. In the diabetic rats, no reduction in the glucose-6-phosphatase activity of liver was observed following eight days' administration of BZ-55; indeed, a rise was evident (figure 4). With the same period of administration of BZ-55 the normal, non-

diabetic rats showed good reductions in glucose-6-phosphatase activity. The rise in glucose-6-phosphatase activity after alloxan, previously reported by other groups,[6, 7] was observed also. The results of this experiment indicate that insulin is required for the effect of BZ-55 on the glucose-6-phosphatase activity of liver, but whether or not an increased amount of insulin is necessary for this effect is not known at present.

Another effect of BZ-55 which has been noted previously is its influence on the thyroid. The administration of BZ-55 was observed to cause a reduction in I^{131} uptake by the thyroid gland and, over a period of time, an increase in its size. Dr. J. Logothetopoulos has compared BZ-55 and Orinase with respect to I^{131} uptake and goitrogenic effect. He found the reduction in I^{131} uptake to be much more pronounced in rats treated with BZ-55 than in those treated with equimolecular quantities of Orinase. The goitrogenic effect of BZ-55 was high also, whereas Orinase in similar doses did not have a goitrogenic effect. In his series too, the hypoglycemic effects of these materials appeared to be unrelated to their effect on the thyroid gland.

It is difficult to interpret the various results. We can say, however, that the results presented seem to be consonant with a stimulation of insulin secretion by BZ-55. It is evident too that the presence of insulin is required for at least one of the changes in the activity of the liver, namely the effect on glucose-6-phosphatase. However, the fact that BZ-55 does not appear to stimulate glucose uptake or glycogen formation in muscle is against this view unless this drug, in addition to stimulating insulin secretion, also has an effect which masks the action of insulin on muscle.

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Studies of the Effect of Carbutamide on Glucose-6-Phosphatase

Carl A. Kuether, Ph.D., Earl G. Scott, A.B., Carlotta Martinez, A.B., Henry M. Lee, Ph.D., and C. W. Pettinga, Ph.D., Indianapolis

In a previous publication[1] it was shown that addition of carbutamide (Amino-phenurobutan, Lilly) to a rat liver microsome preparation in vitro had no significant effect on the glucose-6-phosphatase activity of the microsomes. At the time this work was completed the report of Hawkins and others[2] appeared, showing that oral administration of carbutamide to rats over a period of three weeks led to a decreased glucose-6-phosphatase activity in the liver. The above studies were extended therefore to include the effect of oral administration of carbutamide on the glucose-6-phosphatase activity of the liver of fed and fasted normal and alloxan diabetic rats. This report is concerned with these findings.

From the Biochemical Research Department, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

MATERIALS AND METHODS

Male, 150 to 200 gm. albino rats were made diabetic by the intravenous injection of 40 mg. per kg. of alloxan after a forty-eight hour fast. One week following the injection, all of the animals used for the experiments had blood glucose concentrations (not fasted) of 290 mg. per 100 ml. or more.

Incubations and analytical procedures were performed as described previously[1] except that whole liver homogenate was used as the source of glucose-6-phosphatase.

A group of four, sixteen hour fasted animals was

TABLE 1

Summary of results

Time min.		Weight of rat gm.	Weight liver gm.	Blood sugar change mg. per cent	Blood carbutamide γ/ml.	Liver carbutamide γ/gm.	Liver glucose-6-phosphatase mg.P/gm./15 min.
Group I	0	180	6.2	–	0	0	4.15
Fasted	30	155	5.9	+15	149	96	4.10
normal	60	164	6.1	–10	187	110	4.21
rats	90	170	6.6	–14	194	124	4.23
	120	163	5.9	– 2	144	102	4.72
							4.28±.11*
Group II	0	165	7.5	–	0	0	3.19
Fed	30	155	6.8	–24	144	35	2.97
normal	60	145	5.9	–43	207	48	2.87
rats	90	155	6.9	–59	162	43	3.45
	120	145	5.7	–60	273	62	3.29
							3.15±.10*
Group III	0	150	6.5	–	0	0	4.51
Fasted	30	126	5.1	– 2	115	94	4.63
alloxan	60	145	6.6	+16	125	58	4.68
rats	90	157	6.9	–18	120	52	4.97
	120	162	8.4	–30	156	76	4.27
							4.61±.11*
Group IV	0	180	8.8	–	0	0	4.75
Fed	30	188	7.9	+112	77	28	5.18
alloxan	60	190	9.6	–24	121	54	4.84
rats	90	188	9.4	0	117	40	4.80
	120	182	8.8	+156	96	49	4.49
							4.81±.11*

* Mean and standard error of mean.

given, by stomach tube, 100 mg. per kg. of carbutamide as a 1 per cent aqueous solution. A control animal that had received distilled water instead of carbutamide was killed immediately after dosing; other animals were killed after 30, 60, 90, and 120 minutes. Blood sugar concentrations were determined before dosing and just before killing. Blood and liver concentrations of carbutamide were measured at the time the animals were killed.

RESULTS AND DISCUSSION

The results are summarized in table 1. It will be seen that the dose of carbutamide was sufficient to produce a significant concentration of the drug in the blood and in the liver at 30 to 120 minutes after dosing.

The glucose-6-phosphatase activity of the liver did not change significantly during the first 120 minutes following administration of the drug in any of the four groups of animals. In the group of fed normal rats (Group II) there was a significant drop in blood glucose during this time. The blood sugar changes observed in the diabetic rats were inconsistent, as were those of the fasted normal rats. There does not appear to be any correlation between blood sugar changes and liver glucose-6-phosphatase activity following oral administration of carbutamide.

Mean values of the glucose-6-phosphatase activity were calculated for each group of animals (table 1). In Group II (fed normal rats), the activity was significantly lower than that observed in the other groups. The P value for the difference from each of the other means individually is less than 0.001, indicating a high degree of significance. This is in agreement with the findings of Ashmore and others[3] that fasting and alloxan diabetes both increase glucose-6-phosphatase activity.

SUMMARY AND CONCLUSIONS

Fed and fasted normal and alloxan diabetic rats were given carbutamide in doses of 100 mg. per kg. orally, and were killed after 0, 30, 60, 90, and 120 minutes. At no time was there a significant change in the glucose-6-phosphatase activity of a liver homogenate even though there were significant changes in blood glucose concentrations within this time. Change in liver glucose-6-phosphatase activity is not the cause of the change in blood glucose concentration following oral administration of carbutamide.

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Compounds Inhibiting Insulin Degradation

Robert H. Williams, M.D., Seattle*

Since insulin is rapidly degraded in the body,[1,2] any safe measure employed to prevent this in diabetic patients might be of distinct advantage, particularly since it would probably increase the effectiveness of the body's homeostatic mechanisms. Using an in vitro system previously described,[3] many compounds have been tested for their capacity to inhibit the degradation of insulin. Since most of these studies have been published recently in this *Journal*[4] only a very brief account is given here.

In table 1 is presented the activity of a few representative compounds. It is noted that a wide variety of chemicals cause inhibition of insulin degradation in vitro. Whereas bis (carboxyaminopropionylphenyl) disulfide is highly active, relatively high concentrations are required for some of the other compounds, including carbutamide (butylaminobenzenesulfonylurea). Despite the high in vitro activity of the disulfide compound, it has been found to cause no hypoglycemia in rats. Moreover, carbutamide causes marked hypoglycemia in doses smaller than are apparently required to cause significant inhibition of insulin degradation.[5] Other compounds that are active by the in vitro test, but which have been found by my collaborators and me to cause little or no hypoglycemia in rats, include cystine, dithiouracil 6-ethoxybenzothiazolesulfonamide, 2-acetylamino-1, 3, 4-thiadiazole (Diamox), arbutin, ergothioneine, hydroxyindoleacetic acid, dihydroxyphenylalanine, thiolhistidine, dithioaniline, and bis (morpholinothiocarbamoyl) disulfide.

The fact that there are relatively many compounds which inhibit the degradation of insulin in vitro, many of which do not exert a hypoglycemic effect, poses some important questions: (a) What are the mechanisms by which insulin degradation are accomplished? (b) What are the best procedures for evaluating potential hypoglycemic agents?

TABLE 1

Inhibition of insulin degradation in vitro

	mM/liter causing 50 per cent inhibition
Disulfides	
Bis-β(2-carboxyaminopropionylphenyl) disulfide	.002
Bis-(dimethylcarbamyl) disulfide	.011
Cystine	.1
Lipoic acid	1.1
Thioureas	
2, 4 Dithiouracil	0.5
1-p-Methoxylbenzyl-3-thiosemicarbazide	10
Thiazoles	
2-Amino-5-(p-methoxyphenyl)-1, 3, 4-thiadiazole	0.8
Thiamine	15
Sulfonamides	
6-Ethoxy-2-benzothiazolesulfonamide	0.5
2-Acetylamino-1, 3, 4-thiadiazole	6
2-Isopropyl-5-p-aminobenzene-sulfonamido-1, 3, 4-thiadiazole	11.5
1-n-Butyl-3-p-aminobenzene sulfonylurea	15
Nonsulfur Compounds	
Decamethylenediguanidine	5
Indole-3-propionic acid	7
Arbutin	9
Indole	11
3-Phenylpropionic acid	22

The in vitro inhibitors may react in the following ways: (a) Oxidize the SH-groups of the liver enzyme preparation, (b) combine with an enzyme at "non-key" or "key" sites, (c) combine with insulin, or (d) combine with an insulin-enzyme complex. Enlightenment relative to the types of reaction of different compounds occurring in the liver enzyme-insulin system might

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significantly aid the search for orally effective antidiabetic compounds.

Most of our hypoglycemic tests have been conducted with intact adrenalectomized rats, some of which were primed with glucose during the test. Pertinent questions relative to this type of screening procedure are: (a) Is the rat the best species? (b) Are intact or adrenalectomized animals better? (c) Is it preferable to administer glucose during the test with the objective of stimulating the secretion of insulin? (d) Should alloxanized animals be tested? (e) What are the intervals at which blood glucose should be measured? Presumably a variety of these tests should be employed since they test different phases and since some compounds can be expected to act through different mechanisms.

The clinical results with the sulfonylureas demonstrate that oral therapy can be effective in many diabetic patients. However, the association of the sulfonylureas with significant untoward reaction in some subjects prompts search for still more useful compounds.

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Effects of Substituted Sulfonylureas on Rat Diaphragm and Liver Tissue

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A previous communication from this laboratory[1] reported hepatic changes following administration of the substituted arylsulfonylureas both in vivo and in vitro.

Since glucose-6-phosphatase appears to play a significant role in hepatic glucose production (elevated in diabetes and fasting and deficient to absent in the common form of glycogen storage disease), this enzyme was studied in rat liver. It was found that addition of both compounds in 10^{-2} molar concentration [N-Sulfanilyl-N^1-butyl-urea and N-(4-Methyl-benzolsulfonyl)-N^1-butyl-urea)] to experimental liver preparations in vitro resulted in 25 per cent inhibition of enzyme activity. No inhibition was observed at lower concentrations. This inhibition occurred in both crude homogenates and the separated microsomal fraction. Since inhibition was also observed with sulfanilamide in the same concentration, it was concluded that the sulfonylureas had no direct physiological effect on glucose-6-phosphatase.

Rat liver slices incubated in the presence of these agents showed no change in either the degree of glycogenolysis or the rate of glucose production, nor were any alterations noted in the distribution of labeled products after incubation with uniformly labeled fructose-C^{14} or pyruvate-2-C^{14}.

To study the effects in vivo, rats were fed a single dose of sulfonylurea by stomach tube, killed two to four hours later during the hypoglycemic episode, and the livers assayed for glucose-6-phosphatase activity. No changes were found in the level of this enzyme although blood glucose fell 40 per cent. If the drugs were given every twelve hours for forty-eight hours, however, a marked decrease in hepatic glucose-6-phosphatase activity was noted similar to that found in normal animals treated with protamine zinc insulin over the same period of time (table 1).

From other studies[2] it has become apparent that the level of glucose-6-phosphatase merely mirrors the degree of sustained hepatic glucose production. The enzyme is present in considerable excess when determined

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TABLE 1

In vivo effects of sustained sulfonylurea and insulin administration on hepatic glucose-6-phosphatase activity (after Ashmore et al.[1])

	Animals	Glucose-6-phosphatase mg. P/gm./30 min.
Normal	6	5.5 ± 0.2
Sulfonylurea treated	6	3.5 ± 0.4
Insulin treated	6	3.3 ± 0.5

TABLE 2

Rat hemidiaphragms incubated ninety minutes in Ringer-bicarbonate medium with 10 micromoles/ml. of glucose. Values expressed as micromoles glucose disappearing from the medium per 100 mg. diaphragm dry weight.

Insulin milliunits/ml.	Animals	Insulin added	Control	Per cent change with S.D.
25	4	28.0	15.2	+52 (±19)
2.5	4	28.5	19.6	+51 (±35)
0.25	5	15.0	14.1	+4 (±18)

TABLE 3

As in table 2 except both hemidiaphragms incubated in the presence of insulin and one with 50 mg. per cent sulfonylurea.

Insulin milliunits/ml.	Animals	Insulin plus sulfonylurea	Insulin alone	Per cent change with S.D.
25	3	22.5	22.2	+1 (±10)
2.5	6	21.7	22.7	–3 (±15)
1.0	3	17.6	18.0	–3 (±15)
0.25	6	19.2	18.3	+8 (±26)

directly in comparison to the other glycogenolytic and glycolytic enzymes. Also, after glucagon or epinephrine, its activity is unchanged, even though glucose production is acutely increased many fold. The finding of a reduced level of glucose-6-phosphatase after prolonged treatment with the sulfonylureas represents a diminished hepatic glucose output similar to that seen in the hyperinsulinized animal.

It may be inferred therefore that the sulfonylureas cause the pancreas to secrete more insulin, or facilitate its action, or inhibit its destruction.

In spite of numerous reports which have stated that these agents have no effects on the diaphragm in vitro, a systematic study was undertaken to see if the action of a subthreshold level of insulin could be augmented. Diaphragms from 150 gm. female Wistar rats were removed, trimmed, and soaked for fifteen minutes in a cold Ringer-bicarbonate buffer (containing Na—146, K—5, Ca—1, Mg—0.5, Cl—114 and HCO_3—40 mM/L.) with 10 μM/ml. glucose. Each diaphragm was then divided in two and the paired hemidiaphragms placed in flasks containing 2 ml. of fresh buffer with and without sulfonlyureas in a concentration of 50 mg. per cent, gassed with 95 per cent O_2:5 per cent CO_2, and incubated with shaking for ninety minutes at 37°C. At the end, the tissues were placed in weighing bottles and dried for two hours at 110°C. Medium glucose was then determined by the Nelson-Somogyi method[3, 4] and glucose uptake (μM/100 mg. dry weight) calculated.

In table 2, it is noted that paired hemidiaphragms show a 50 per cent greater glucose uptake with insulin concentrations of 25 and 2.5 milliunits per ml. No effects were observed with 0.25 milliunits/ml. In table 3, paired hemidiaphragms have been incubated in the presence of insulin with and without the sulfonylureas. At all insulin concentrations, both above and below threshold, no enhancement of insulin action was observed.

It can therefore be inferred that not only do the sulfonylureas fail to have a direct effect on glucose uptake by the rat diaphragm, but they also do not facilitate or augment the action of what insulin is available, even when the insulin is present in barely subthreshold amounts.

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Lack of Effect of Carbutamide (BZ-55) on the Metabolism of Alcohol

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The reactions by which the body metabolizes over 90 per cent of absorbed ethyl alcohol occur in three stages. These are: alcohol to acetaldehyde; acetaldehyde to acetate, and acetate to carbon dioxide and water. Considerable evidence has accumulated indicating that the chemical reactions involved are associated, in some as yet poorly understood manner, with the metabolism of carbohydrates. The administration of glucose with or without insulin has been reported by some to accelerate Stage 1, but others have not been able to confirm these reports. Several substances (disulfiram, cyanamide, etc.), definitely inhibit Stage 2 and at the same time increase the toxicity of alcohol. Little is known concerning the factors which might influence Stage 3.[1,2,3] Since BZ-55 lowers blood glucose we investigated what effects, if any, it might have on the metabolism of alcohol.

The rate of disappearance of alcohol from the blood and changes in blood acetaldehyde, blood pyruvate and blood glucose were studied in six dogs following test doses of alcohol before, during, and after oral BZ-55 medication. The dogs were male mongrels weighing approximately 10 kg. each. The test dose of alcohol was 1 gm. per kg., diluted one to three with normal saline, and injected intravenously over a ten-minute period. Blood was obtained for analysis just before and at 45 minutes, 2, 3, 4, 5, and 6 hours after the alcohol infusion.. The methods employed for the analyses were: alcohol, Harger;[4,5] acetaldehyde, Stotz;[6] pyruvic acid, ethyl acetate extraction, Friedemann and Haugen;[7] glucose, Folin and Malmros.[8] Each dog was studied four times as follows: 1) control, no medication; 2) after BZ-55 medication for one week; 3) after double the dose of BZ-55 for the following week, and; 4) one week after the drug was discontinued.

Two dogs (423 and 424) received by mouth 0.5 gm. BZ-55 twice daily for the first week and 1 gm. twice daily for the second week. The resultant blood levels of BZ-55 were fairly constant and proportional to the dose. Dog 423 had a blood level of about 25 mg. per cent with the smaller dose and about 50 mg. per cent with the larger. The blood concentrations of BZ-55 for Dog 424 for the same periods were 15 and 25 mg. per cent. Dogs, 3, 4, 5 and 6 were given one-half as much BZ-55 as dogs 423 and 424, and blood levels of 7 to 10 for the first, and 25 mg. per cent for the second week were obtained. BZ-55 was found to have disappeared from the blood in one week after the last dose. The dogs lost from one to three pounds during the tests but appeared healthy with no loss of appetite. Dog 423 exhibited considerable diarrhea by the end of the second week on BZ-55.

Figure 1 shows the rate of disappearance of alcohol (Stage 1) from the blood stream of each of the six dogs. Although there were variations from week to week and from hour to hour, the slope of the curves are the same with and without BZ-55 medication. This identity in slope is more clearly shown by plotting mean values for all control and for all treatment periods (figure 2).

In table 1, the average blood concentrations for acetaldehyde, pyruvate and glucose are given. Only the control values and those found at 45 minutes and two hours after the injection of alcohol are given. The findings at three, four, five and six hours were not different from those reported, except that the moderate elevation of acetaldehyde which occurred after test doses of alcohol gradually returned to control levels. The results of analyses at the forty-five minute and the two hour intervals are important because it is at this time that any antabus-alcohol-like reaction would be detected by increases in blood acetaldehyde. It is evident that the increase in blood acetaldehyde which followed the test doses of alcohol are not significantly different during BZ-55 administration from those obtained during the control periods. At no time were the blood acetaldehyde concentrations indicative of an antabus-alcohol-like reaction, and none of the animals exhibited any intolerance to alcohol. The blood pyruvate levels during treatment with BZ-55 were lower than those found during the control periods. However, all of these values are within the range which we find for normal dogs. Blood glucose was lowered about 20 mg. per cent during the weeks the dogs received BZ-55, but there was no change in blood glucose correlated with the test doses of alcohol.

SUMMARY

Daily oral dosage with carbutamide for as long as two weeks had no effect on the rate of disappearance of alcohol from the blood (Stage 1); did not increase blood acetaldehyde after test doses of alcohol (Stage 2, antabus-alcohol effect); or change blood pyruvate levels

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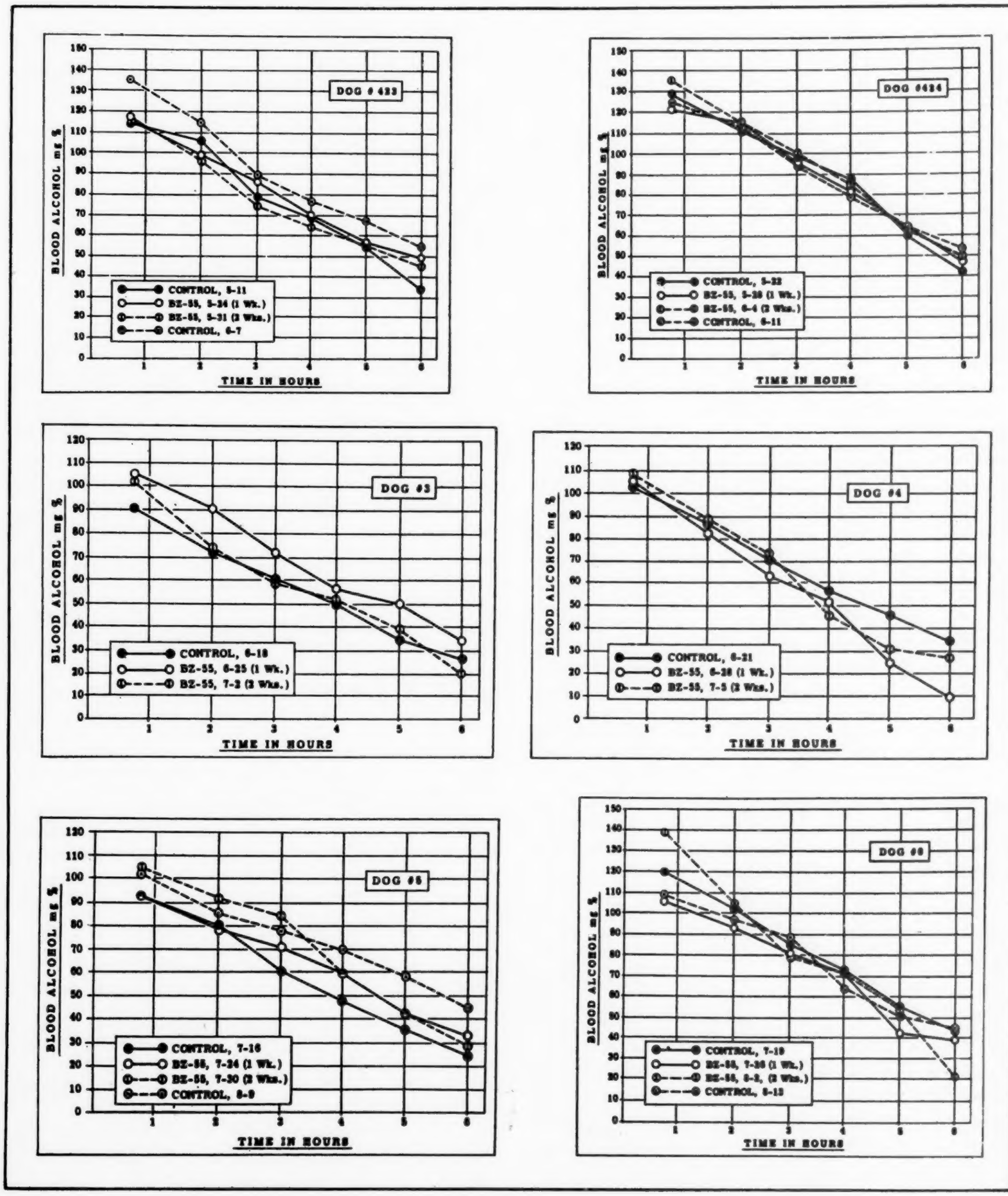

FIG. 1. Curves representing the rate of metabolism of alcohol (Stage 1) in six dogs, before, during and after treatment with BZ-55. The points on these curves show the concentration of alcohol remaining in venous blood. The dose of alcohol in each case was 1 gm. per kg. given intravenously.

from the normal range. Blood glucose was reduced about 20 mg. per cent while the dogs were receiving carbutamide. There was no relationship between this lowered blood glucose and the administration of alcohol.

TABLE 1

Comparison of average values for blood acetaldehyde, pyruvate and glucose, before and after a test dose of alcohol during control and BZ-55 treatment periods.

	Untreated (control) blood levels mg. per cent			Treated (BZ-55 for 1 and 2 wks.) blood levels mg. per cent		
		Time after alcohol			Time after alcohol	
Substance	Before alcohol	45 min.	2 hours	Before alcohol	45 min.	2 hours
Acetaldehyde	.017 (.001-.061)	.041 (.012-.092)	.045 (.012-.165)	.017 (.001-.025)	.058 (.001-.123)	.051 (.001-.103)
Pyruvate	1.31 (.92-1.98)	1.23 (.82-1.90)	1.16 (.98-1.40)	1.17 (.82-1.62)	.94 (.56-1.40)	.88 (.72-1.12)
Glucose	96 (90-102)	100 (92-104)	98 (88-108)	84 (72-99)	83 (76-88)	80 (72-88)

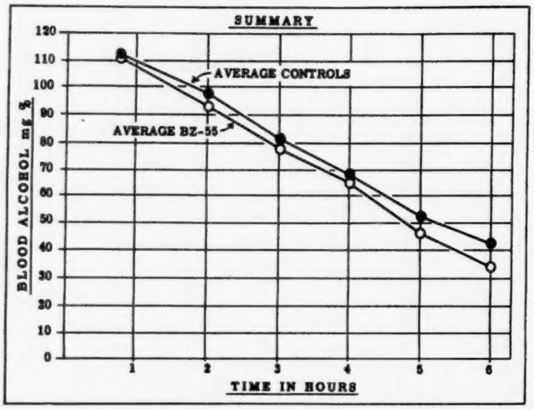

FIG. 2. Comparison of the rate of disappearance of alcohol in dogs, with and without BZ-55 medication. These curves were obtained by averaging the data shown in figure 1.

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Group Discussion

JAMES B. FIELD, M.D., (*Bethesda, Maryland*): I would like to present table 1 (reprinted with permission from *Proceedings of the Society of Experimental Biology and Medicine*) in confirmation of the findings of Dr. Cahill on the rat diaphragm. We were interested to test if carbutamide itself has an insulin-like action on the diaphragm or if it augments the action of insulin. Our system is similar to Dr. Cahill's except that we measured glycogen deposition instead of glucose disappearance. One can see that at approximately 10^{-2} molar concentration of carbutamide that there is no difference from the control diaphragm without the drug. We concluded on the basis of these experiments that carbutamide had no insulin-like action to augment glycogen deposition in rat diaphragm.

The next column merely shows the known insulin effect in increasing the deposition of glycogen. When one hemidiaphragm is exposed to 0.2 unit of insulin and 2 mg. of carbutamide, and the other hemidiaphragm exposed only to carbutamide, we found a significant inhibition of the insulin effect. The point that I would like to make here is that at this concentration, the inhibition of insulin action is probably the same sort of nonspecific inhibition that we heard about this morning in respect both to insulinase and to glucose-6-phosphatase. I think that Dr. Haist has reported that

TABLE 1
The effect of insulin and carbutamide on glycogen deposition of rat hemidiaphragm

Material used with treated diaphragm	Treated Diaphragm micromoles (glucose equivalent) per gm. tissue Mean ± S.E.M.	Control Diaphragm micromoles (glucose equivalent) per gm. tissue Mean ± S.E.M.	Insulin Effect micromoles (glucose equivalent) per gm. tissue Mean ± S.E.M.
*Carbutamide 2 mg.	16.63±0.74	16.29±0.90	0.34±0.76 (6)†
Insulin .2 unit	23.54±0.97	17.24±0.82	6.29±0.59 (25)†
Insulin .2 unit + carbutamide 2 mg.	18.50±0.95	15.60±1.05‡	2.90±0.70 (18)
Insulin .2 unit + carbutamide .2 mg.	15.65±1.71	10.74±1.26‡	4.91±0.74 (9)
Insulin .2 unit + sulfadiazine 2 mg.	17.37±1.11	15.44±1.69§	1.93±1.35 (6)

* Carbutamide control; no insulin.
† Number of determinations.
‡ Control hemidiaphragm also treated with carbutamide.
§ Control hemidiaphragm also treated with sulfadiazine.

cytochrome oxidase is also inhibited at 10^{-2} molar concentration. When the same experiment was repeated using a concentration of carbutamide in the physiological range (10^{-3} M) one sees that the insulin effect is not significantly different than when no carbutamide is present. Consequently, at a physiological concentration these drugs do not inhibit the insulin. In physiological concentrations no inhibition of glucose-6-phosphatase or change in the glucagon and epinephrine response of liver slices was found. It is important to emphasize this differentiation between the amounts of material used. Many of the inhibitory findings that have been reported could probably be explained upon the basis of the large amount of carbutamide used.

Francis D. W. Lukens, M.D., (*Philadelphia*): I have a question: As I understand it, there is a sharp distinction between the effects of BZ on alcohol and on barbiturates. Is that correct?

Mary A. Root, Ph.D., (*Indianapolis*): On this work on alcohol I have no comment to make. As far as the barbiturates are concerned, my own feeling at the moment is that with low doses of barbiturates (the type that would be taken by patients in the hospital for sedation) we are not getting any measurable effects. When larger doses are given then there is a prolongation of action when giving the animal carbutamide. I wonder if whatever the effect of carbutamide on the degradation of barbiturate is, if it isn't something that shows up only when the system for degrading barbiturates is working at full force so to speak, whereas with much lower doses, this won't show up.

Joseph L. Izzo, M.D., (*Rochester, New York*): There have been nine papers this morning, and when you consider these nine papers in conjunction with the discussions and the other available experimental evidence, it seems to me that the positive effects of the sulfonylureas can be narrowed down to two. One, the effect of sulfonylureas on the pancreas, and two, the effect of sulfonylureas on the liver. Are these two independent? Or is the effect on the liver primary? Or is the effect on the pancreas primary, with the secondary effect on one or the other?

There are certain critical lines of evidence that should be explored. First: One critical study is that of Miller and Dulin. If the effect is primarily on the pancreas, then it would be very hard to show why these materials increased liver glycogen rather than muscle glycogen. Second: I do not think that Dr. Lukens' work agrees with the primary effect on the pancreas. Third: The effect Dr. Vaughan showed on the inhibition of glycogenolysis by the liver.

M. E. Krahl, Ph.D., (*Chicago*): I would like to make a comment on the papers of Dr. Haist and Dr. Cahill, further substantiating the lack of effects of BZ-55 on peripheral glucose utilization. The first comment is related to glucose uptake by adipose tissue in vitro. At the March meeting I reported a marginal positive effect of BZ-55 when adipose tissue was incubated in normal rat serum. This experiment was repeated incubating the adipose tissue in Krebs' bicarbonate solution instead of serum. There was no effect of BZ-55 on the glucose uptake, and I am inclined to feel that the effect of BZ-55 on adipose tissue is therefore nonexistent.

The second experiment in the same direction comes from a paper sent to me by Dr. Arne Wick, which he has given me permission to quote. He tested the effect of BZ-55 on the blood sugar of eviscerated rabbits, both thin rabbits and very obese rabbits. In neither case did he get a hypoglycemic effect of BZ-55. Furthermore, in agreement with Dr. Cahill's results on diaphragms, he did not get a potentiation of submaximal amounts of insulin. So taken together, this adds further weight to the absence of a peripheral BZ-55 effect.

Martha Vaughan, M.D., (*Bethesda, Maryland*): I

would like to disagree with one point which Dr. Field made and to clarify the matter of concentrations of Orinase which we used to obtain an inhibition of the epinephrine effect. We obtained about 80 to 90 per cent inhibition of the glucagon or epinephrine effect with 5 x 10^{-3} molar and have been able to show about a 40 per cent inhibition of the epinephrine effect on liver slices at 5 x 10^{-4} molar Orinase. These concentrations I think were well within the range of what one might expect from the blood levels which have been measured.

WILLIAM C. STADIE, M.D., (*Philadelphia*): I'd like to draw attention to the situation that existed during early studies of acetylcholine when its enzymatic hydrolysis was under intensive investigation. These early studies always referred this to the action of cholinesterase, but subsequently it was found that there were a good many esterases quite nonspecific, but there was only one cholinesterase. Technics were devised to distinguish them. I wonder, Dr. Williams, if somewhat the same situation doesn't exist now with respect to insulinase. I refer to the fact that you find so many compounds with quite divergent structure but having the same ability to inhibit "insulinase." Perhaps you are dealing with many different proteinases.

ROBERT H. WILLIAMS, M.D., (*Seattle*): This is a problem that is too complex for me to answer properly, except I would like to say that we feel that much more is involved than a single "insulinase," and for that reason we try to avoid the use of the term. In fact, we already have a good deal of specific information to indicate that more than one enzymatic activity is involved.

R. E. HAIST, M.D., (*Toronto*): I would just like to point out a difference between our experiments and those of Dr. Kuether, namely that the dose was five times as large—500 mg. per kg. of body weight. I don't think that the effect can be due to undernutrition because the animals in our series were pair-fed. This brings up a point. It seems to me that there is no such thing as a physiological dose of BZ-55; the dose that one uses is purely arbitrary, the dose which will produce an effect.

DR. MARY ROOT: Dr. Haist, when you were studying the effect on pancreas and islet tissue, you showed that in the animals treated with BZ-55 there was a decrease in the weight of the pancreas. In a few dog experiments I have done, we found a small but definite decrease in the weight of the pancreas removed from the dogs that had been treated with BZ-55. I had no idea what this meant nor any explanation for it. I didn't mention it before, but in conjunction with your work I think that it may have some importance.

ALBERT E. RENOLD, M.D., (*Boston*): I would like to ask Dr. Forney to what extent the disappearance of alcohol differs when insulin or carbohydrate is administered rather than carbutamide?

HAROLD R. HULPIEU, PH.D., (*Indianapolis*): In the few experiments that we have done with insulin and with insulin plus glucose in normal animals we can not see any change in the disappearance rate. I know that is contrary to several other published papers. We do not find that insulin or insulin plus glucose in a normal animal changes the disappearance rate whatsoever.

DR. WILLIAMS: I would like to discuss some of the combined observations of Dr. Haist and Dr. Mary Root in relation to what we know to be the situation in regard to the thyroid. Dr. Haist has demonstrated an increase in the islet cell mass. Dr. Root has demonstrated a decrease in the insulin concentration in the pancreas. Are those observations correct?

DR. HAIST: Those observations are correct, but they apply to different species, and that's the joker in this particular series of experiments. The two species react differently.

DR. WILLIAMS: In the case of the thyroid one can get a marked increase in the cell size and a decrease in the thyroid hormone, and in one instance it may be associated with thyrotoxicosis and another instance with myxedema. This illustrates the importance, if it is possible for us to know, of the insulin content of the plasma on the effect of the sulfonylureas.

GEORGE F. CAHILL, JR., M.D., (*Boston*): I don't know whether this is correct, but I'd like to ask Dr. Lukens: Dr. Vallance-Owen, who I believe worked in his laboratory, has a superb system now for measuring serum insulin using the rat diaphragm. I wonder if he has measured serum insulin in animals treated with the sulfonylureas.

DR. LUKENS: That has not been done.

DR. HAIST: I entirely agree that if we could use some method that would show us whether or not the islets are secreting insulin under a certain set of circumstances, that would be grand.

DR. LUKENS: In connection with that, the collaborators of Dr. Foa came to Philadelphia and learned Dr. Vallance-Owen's method. If they use the blood from the pancreatic vein of the animal treated with BZ-55 to study directly a possible increased insulin output, they will have what's probably the most sensible attack we could make on this problem.

The Site of Action of the Arylsulfonylureas in Man

A. E. Renold, M.D., A. I. Winegrad, M.D.,† E. R. Froesch, M.D.,‡ and G. W. Thorn, M.D.,§ Boston*

The data presented in this report had already been submitted for publication at the time of the Lilly Symposium.[1] To facilitate the understanding of references to this work which appear in the discussion, a brief summary of the results follows.

Metabolic balance studies and comparative intravenous tolerance tests with glucose, fructose, and galactose have been carried out before, during, and after therapy with the arylsulfonylurea compounds. These studies were carried out in normal subjects and in patients with diabetes mellitus. Particular emphasis was given to a study performed in a patient with diabetes mellitus who had undergone hypophysectomy two months previously. It was hoped that the metabolic effects of these agents might be better demonstrated in a patient in whom several endocrine homeostatic mechanisms had been replaced by controlled hormone administration.

The hypophysectomized diabetic patient showed a good but not an excessive response to both the aminobenzene and the toluene derivatives. In addition the compounds were shown to be without effect on the urinary excretion of 17-hydroxycorticoids. The aminobenzene, but not the toluene derivative, decreased the thyroidal uptake of I^{131}. Despite this last observation the conclusion was reached that the hypoglycemic effects of the arylsulfonylureas are not primarily due to alterations in pituitary, adrenal, or thyroid function.

Failure to demonstrate significant effects of these compounds on the rate of blood glucose removal after a glucose load, as well as failure to obtain appropriate changes in serum or urine phosphate, in blood lactate and pyruvate, or in the urinary excretion of nitrogen suggest that an "insulin-like" effect on peripheral glucose utilization is probably not a major component of the mode of action of the arylsulfonylureas.

The effects of the arylsulfonylureas upon hepatic gluconeogenesis were evaluated by means of comparative glucose, galactose, and fructose tolerance tests with particular emphasis on the conversion of galactose and fructose to glucose. The administration of the arylsulfonylureas resulted in decreased conversion of both fructose and galactose to glucose and this was interpreted as suggesting decreased hepatic glucose release or synthesis. Although no demonstrable hepatic dysfunction appears to result from the administration of these compounds the evidence presented suggests that selective interference with hepatic gluconeogenesis might significantly contribute to their hypoglycemic action.

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Observations on the Action of Tolbutamide

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The arylsulfonylureas seem to produce their hypoglycemic effects in the main by inhibiting hepatic glycogenolysis.[1] There is little evidence that they mimic the action of insulin in the periphery[2] in the sense of increasing the transport of glucose across cellular membranes or of promoting its phosphorylation in the tissues. Insulin, on the other hand, in addition to exercising both of these peripheral functions also blocks the excessive hepatic glycogenolysis which characterizes the uncontrolled diabetic state. Since on administration of the sulfonylureas a fall in peripheral blood glucose is in evidence *only* if insulin is present in the organism,[3] these drugs cannot reasonably be construed as being capable of substituting for insulin. The pharmacologic limitations of the sulfonylureas can be brought into focus by comparing their actions in normal and in totally depancreatized dogs in respect to their effects (1) on the utilization of glucose by peripheral tissues as this is thought to be reflected in changes in a-v difference in the extremities, and (2) on hepatic glycogenolysis. The authors have studied tolbutamide (Orinase) in these parameters. In addition, the influence of the drug on the body's sensitivity to insulin and to glucagon has been explored.

Change in a-v Difference

Procedure 1 (in a normal dog): After a control period of ten days during which time a normal dog was fed a free mixed diet including one to two pounds of liver daily, the animal was heavily ergotaminized (dihydroergotamine 0.5 mg./kg. i.v.) fourteen hours postabsorptive. Under pentobarbital anesthesia, simultaneous arterial and venous blood glucose* determinations were made from an extremity at one-minute intervals (with due correction having been made for the venous circulatory lag.† The plotted curves of these two sets of readings served to establish predrug a-v difference (figure 1a). For the next twenty-two days under the same dietary conditions, the animal received with his food a daily dose of D860 (Orinase). During the last fourteen days of this period the dose was fixed at 200 mg./kg./day. The blood sampling was then repeated under the same experimental conditions (figure 1b).

Findings:

After the course of Orinase, there was noted a marked lowering in all blood-glucose determinations. In addition, the mean of all arterial readings (which had in the predrug curves been higher than the venous mean) became practically identical with the venous mean, arteriovenous difference becoming practically negligible as compared with this difference before the administration of Orinase. Promptly after starting heavy dosage of the drug (200 mg./kg./day), the animal became ravenously hungry and continuously lost weight despite a markedly increased food intake. With this loss of weight, there occurred a progressive increase in behavior irritability, intentional tremors and ataxia noted especially on walking and in seizing food.

The same procedure was carried out in a second animal with similar reduction in peripheral blood-glucose levels as well as in a-v difference.

The increase in appetite which promptly occurred with large dosage of Orinase suggested that the finding of a diminishing a-v difference might be related to the excessive urge for food[4] and that the diminished a-v difference might be detected much earlier than in days of exposure to the drug. It was subsequently noted that in the intact dog under heavy dosage of Orinase-sodium by vein (even a single dose of 100 mg./kg.) arterial blood glucose as well as a-v difference fell within

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The glucagon and glucagon-free insulin used in this research were supplied by Eli Lilly and Company through the courtesy of Drs. Franklin B. Peck, Sr., and William R. Kirtley. Orinase-Upjohn was supplied by Upjohn Company through the courtesy of Dr. C. J. O'Donovan.

*By Somogyi-Nelson macromethod.

†By timing the blood circulation between the artery and the point of venous sampling with fluorescine under a Wood lamp.

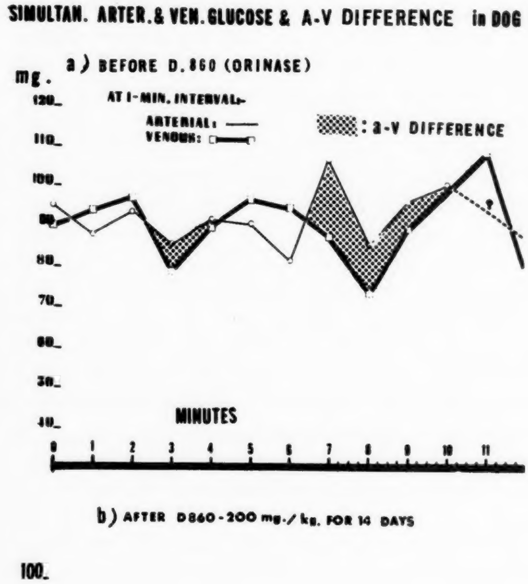

FIG. 1. Simultaneous femoral arterial and venous blood glucose[14] at one-minute intervals (corrected for venous lag) in a normal dog under pentobarbital anesthesia, fourteen hours postabsorptive: (a) before administration of Orinase (b) twenty-two days after administration (200 mg./kg./day—fourteen days). Note in (b) the lowering of both arterial and venous levels and the reduction in a-v difference after exposure to drug.

ten to sixty minutes after administration.

Procedure 2 (in depancreatized dog): A normal dog was pancreatectomized (totally as ultimately proved by postmortem examination). One week was allowed for recovery from the surgical procedure. During this time no effort was made to keep the animal aglycosuric; in fact, only sufficient crystalline insulin was given to keep the dog out of overwhelming ketosis. Then, after heavy ergotaminization under pentobarbital anesthesia, the blood sampling was carried out as in the first procedure, whereupon Orinase-sodium (100 mg./kg.) was given by vein over a five-minute period. Simultaneous arterial and venous samples were again taken.

Findings:

In keeping with the uncontrolled diabetic state of the animal, at the time of the predrug sampling (fifteen hours after the last small dose of crystalline insulin), the dog was approaching ketosis (figure 2). Glucose determinations made twenty-two minutes after the injection of Orinase showed no significant change in a-v difference incidental to the drug. After a postdrug control-run of blood sampling, a small dose of glucagon (0.15 μg./kg.) was given by vein. This promptly (within three minutes) caused an extensive rise in arterial glucose. Venous glucose, however, rather faithfully (almost in parallel) followed the rising arterial curve. Again, there was no significant change in a-v difference such as would have been anticipated if the animal had been intact so far as his pancreatic function is concerned. (With the pancreas intact, the increased arterial glucose gradient incidental to the glucagon administration would have served to widen rather than narrow a-v difference.)

At the 43-minute point (figure 2) while arterial glucose was still rising as a result of the injected glucagon, a small dose of glucagon-free insulin (0.1 unit/kg.) was administered by vein in zero time. Within one and

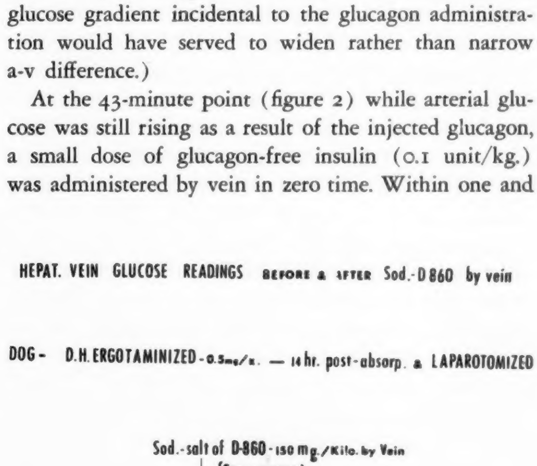

FIG. 2. Hepatic venous glucose (direct needling) determinations in normal dog under pentothal at one-minute intervals be!o e and after heavy dosage of Orinase by vein. Quantitative output of glucose over five-minute periods before and after the drug. Note the effect of Orinase in reducing glycogenolysis within a ten-minute period. (Adapted from a figure in "Proceedings of Society of Experimental Biology and Medicine." Vol. 92, 1956.)

one-half minutes thereafter, the venous glucose level broke away from its parallelism with the arterial curve and precipitously descended with an ensuing marked increase in a-v difference. (This widened difference was presumably the functional expression of the concurrent action of both hormones.)

Comment

Under the conditions of this experiment, Orinase alone, even in large dosage, without the presence of insulin had no apparent ability to increase a-v difference in an extremity.

DISCUSSION

The sharp increase in a-v difference occurring immediately after the injection of insulin bespeaks the markedly increased sensitivity of the organism to insulin, a condition which did not arise from exposition to Orinase. Such change is the direct result of total pancreatectomy[5,6] in any mammalian animal. It has been well documented that the phenomenon of increased sensitivity to insulin occurs consistently in the totally depancreatized dog and man. Nor is the degree of sensitivity to insulin in this dog related to the administered sulfonylurea, since in the totally depancreatized dog not under the drug, the same dose of crystalline insulin produces a similar dramatic fall in venous glucose.

It has repeatedly been demonstrated that the absolute insufficiency commonly associated with the juvenile type of diabetic is attended by a markedly increased sensitivity to insulin.[8,9] This is of clinical significance since we have been able to demonstrate by routinely employing tests for insulin-sensitivity in patients preliminary to Orinase therapy that those who responded well to the drug usually showed the reverse, an impaired or decreased sensitivity[10] to insulin and vice versa. The state of sensitivity did not change perceptibly in any patient during such therapy. Certainly there was no change in the direction of a normal response to the hormone. In contrast to this lack of improvement in insulin-sensitivity in the Orinase-treated subject, the diabetic patient who attains his clinical improvement by diet alone or by diet with insulin promptly shows a progressive improvement in such sensitivity[11] as measured by the conventional test for insulin-sensitivity or by the "six-minute test."*[12] Such improvement in sensitivity is, moreover, usually accompanied by an increase[10] in a-v difference during the state of postabsorption. This finding together with decreased requirements for extrinsic insulin has been interpreted by the authors as indicating an improvement[11] in the economy of insulin in the body. The contrasting lack of effect of Orinase on a-v difference (tissue utilization of glucose) and on improved efficiency of intrinsic insulin is to be emphasized.

Comment

The findings encountered in Procedures 1 and 2 would suggest that the hypoglycemic effect of the sulfonylureas is essentially *not* at the tissue level. If it were, one might reasonably expect to be able to elicit some detectable evidence of an improved insulin-like effect such as the tissue utilization of glucose. Under the conditions of these experiments, Orinase gave no evidence of increased transport of glucose across cellular membranes (fall in venous glucose with increased a-v difference), an important criterion for any peripheral insulin-like function. The hypoglycemic influence of the drug would, accordingly, seem to rest with the restriction of glucose supplied to the arterial stream rather than with the increased clearance from it. There is no similar sparsity of evidence that the hypoglycemic effect is largely central in the liver.

THE INFLUENCE OF ORINASE ON HEPATIC GLYCOGENOLYSIS

(a) *Effects on the Normal Dog*: The inhibition of hepatic glycogenolysis in the nondiabetic dog was reported in detail by the authors in another communication.[1] Briefly, in the postabsorptive state it was found by direct needling of the hepatic vein in a heavily ergotaminized dog under pentobarbital anesthesia that the administration of a single large dose of Orinase-sodium (150 mg./kg. i.v.) causes within ten minutes of its exposition a 28 per cent drop in the output of glucose into the hepatic vein. In the normal dog this reduction in hepatic release of glucose is accompanied by a corresponding drop in femoral arterial glucose and the full train of effects of reduced peripheral a-v difference (already cited).

Whether Orinase has a similar central effect on hepatic glucose output in the depancreatized animal has

* In brief, the test measures the promptness and sharpness of the body's response to insulin administered by vein. An insulin which has been freed of its hyperglycemic-glycogenolytic factor, "glucagon," is used. A single dose of three units of this insulin is given by vein in the fasting state. Venous glucose levels are determined in the fasting state and at two, four, and six minutes after the administration of insulin. The normal individual exhibits within the six-minute period a fall in blood glucose of 19 per cent, with a differential of ± 7 per cent. These criteria have been established by a study of over 600 tests.

since been explored by the authors.

(b) *Effects in the depancreatized dog*

Procedure

A normal dog was pancreatectomized under pentobarbital anesthesia and permitted to recover from the surgical insult without controlling his diabetic state with insulin, save to keep the animal out of fulminating ketosis. After surgical recovery, the dog was again laparotomized (fourteen hours postabsorptively) and blood samples were simultaneously taken at one-minute intervals from the portal vein and from an hepatic vein as well as simultaneously from a femoral artery and vein. Correction was made for the circulatory time lag* between the points of sampling.

After a series of such determinations in order to establish the predrug status of blood glucose at the various points, Orinase-sodium was given by portal vein (150 mg./kg.) over five minutes time. Ten minutes later a similar run of blood sampling was instituted.

Findings:

Ten minutes after completing the injection of Orinase-sodium into the portal vein, there was noted a step-like decline in hepatic venous glucose content, a fall not nearly so dramatic as that observed in the intact dog. Before administration of the drug, the mean hepatic venous level was 212 mg./100 ml. Hepatic venous glucose determinations made at one-minute intervals from the tenth to the nineteenth minute after the drug showed a significant decline: Mean value during the first three minutes of this period was 204.7 mg./100 ml.; during the last three minutes, 194.0 mg./100 ml., a drop of 8.5 per cent from the predrug level in eighteen minutes. There simultaneously occurred a parallel gradient of glucose fall in femoral arterial blood. Both hepatic venous and peripheral values never went below hyperglycemic levels.

Comment:

The arylsulfonylureas seem to block liver glycogenolysis in the totally depancreatized dog precisely as in the normal animal. This block is, however, not clinically discernible in the periphery, presumably because of the hyperglycemia which prevails throughout the entire vascular system. The clearing of this glycemia would seem to depend on removal of glucose in the periphery, which evidently does not adequately take place in the depancreatized animal. The parallelism existing between hepatic venous and femoral arterial decline suggests that any reduction at the latter point depends on restriction of production. In the absence of insulin and, accordingly, of peripheral utilization, there can occur no clinically perceptible hypoglycemic effect from Orinase. This concept may well explain the failure of Orinase to produce its peripheral hypoglycemic effect in the depancreatized or alloxanized dog or clinically in the juvenile diabetic with an absolute insufficiency of insulin.

A POSSIBLE MECHANISM FOR THE BLOCK IN GLYCOGENOLYSIS

The phosphorylase systems in the liver seem to be only indirectly implicated in the central effect of the arylsulfonylureas. Their ability to function remains unimpaired save that they seem to have been deprived of the normal activating factor or trigger mechanism to such function. After Orinase administration, exposure to even a most minute dose of an activating factor such as epinephrine or glucagon provokes an immediate and tremendous response in hepatic venous glycemia. This rise in blood glucose is directly reflected in the peripheral arterial system almost in parallel.[14] It occurs in the depancreatized dog as well as in the normal animal. A minute dose of glucagon in a heavily ergotaminized dog (to block out adrenal-medullary action) calls forth the same glycogenolytic response, which is greatly intensified over that elicited in the same animal before administration of Orinase.

In the Orinase-treated dog, the simultaneous administration of the small dose of glucagon together with a second large dose of Orinase makes no change[1] whatsoever in this increased response to the hormone. Orinase, accordingly, does not seem to interfere with the action of glucagon, once this hormone has been supplied or elaborated. The interference with glycogenolysis seems to be close to the point of elaboration of glucagon. The absence of detectable morphologic change in the alpha cells of the pancreas does not rule out the feasibility of a block in some enzyme system essential to the elaboration of the hormone. The prompt creation by Orinase of increased sensitivity to glucagon both in the dog and in the diabetic human is quite analogous to the organism's acute sensitivity to insulin after pancreatectomy. It could bespeak an absolute lack of intrinsic glucagon ushered in by the drug.

This principle can be applied practically in the sense of predicting which diabetic individuals may reasonably be expected to respond clinically to Orinase. Those diabetic patients who before treatment with the drug are normally responsive or overresponsive to glucagon, in the experience of the authors, usually do not respond satisfactorily to Orinase. By contrast, 80 per cent of those who before treatment were found to be relatively

* Determined by fluorescine under a Wood lamp.

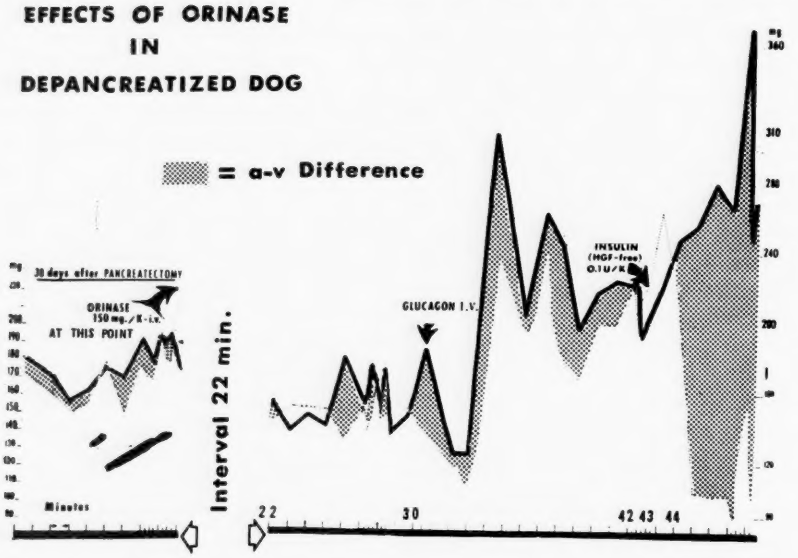

FIG. 3. Arterial curve, heavy line; venous, fine line. Simultaneous arterial and venous[14] blood glucose determinations (corrected for venous lag) in depancreatized dog under pentobarbital anesthesia, fourteen hours postabsorptive. Note small a-v difference (often negative) in this animal before exposure to drug. This was not significantly improved by Orinase in massive dosage or by glucagon alone, but promptly and extensively widened by a small dose of insulin by vein.

insensitive to the hormone had a favorable clinical response to the drug. During treatment with Orinase, sensitivity to glucagon usually increases, only to revert to the original state of insensitivity several weeks after discontinuing the drug. These findings are graphically shown in figure 4.

SUMMARY

The effects of tolbutamide (Orinase) on central and peripheral blood-glucose values were explored in normal and in the depancreatized dogs.

During postabsorption, in the *normal* animal after a large dose of Orinase-sodium by vein, there occurred within ten minutes after its administration a 28.5 per cent drop in the output of glucose from the liver into the hepatic veins. In the normal animal, this fall in glucose output was reflected throughout the peripheral arterial tree as a parallel lowering of blood glucose. With it, there occurred a reduction in peripheral a-v difference down to negligible values.

In the *depancreatized* animal during postabsorption, glucose output by the liver was reduced but not as markedly or as rapidly in the given time as in the normal dog. This milder restriction of hepatic glycogenolysis was also reflected in peripheral arterial blood as a parallel gradient of fall. The lowering of glucose output was, however, not sufficiently marked to clear hyperglycemia at either point. Presumably, a lack of insulin in the organism prevents adequate peripheral utilization of the arterial glucose perfusing the tissues. Such unphosphorylated glucose returns into the venous circulation.

If a-v difference serves as a criterion for the utilization of glucose by the tissues, under the conditions of these experiments, one must conclude that the action of Orinase as measured by this parameter is not one of promoting peripheral utilization of glucose. Any peripheral hypoglycemic effect seems to be directly dependent not on the drug's action at this point but on the peripheral function of insulin. Orinase, therefore, cannot reasonably be considered to act as a substitute for insulin.

Orinase has no influence on the body's sensitivity to insulin. An impaired sensitivity to this hormone, accordingly, does not improve with Orinase as it does by dietetic treatment alone or with insulin.

In both the normal and the depancreatized dog as well as in the clinical diabetic by contrast, promptly after exposure to the drug there occurs a marked increase in the organism's sensitivity (responsiveness) to glucagon. Patients who before treatment with the drug were relatively insensitive to glucagon showed the best clinical response to Orinase and vice versa. Glucagon sensitivity

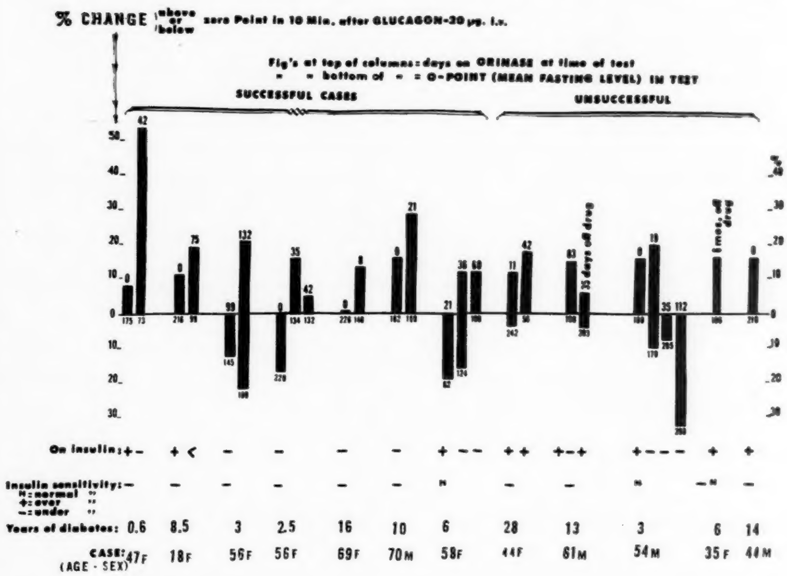

FIG. 4. Black columns indicate in patients on Orinase percentage change above or below the fasting level ten minutes after glucagon (20μg) by vein. Tests for responsiveness to glucagon were routinely performed before, during and after treatment with the drug. Figure at top of each column indicates number of days on Orinase at time of that test; figure at bottom, the mean fasting glucose level at same time.

Note: Those who were successfully treated with Orinase showed little or no response to glucagon before treatment but usually a markedly increased response during treatment.

Test: In the fasting state, with the patient in recumbency, a 20-gauge needle is inserted into an antecubital vein and a slow saline drip attached to keep the needle patent. Six or eight minutes are allowed to elapse for subsidence of any adrenal medullary effect incidental to the needling. Then a heparinized syringe venous blood is withdrawn continuously and evenly over three minutes (by stop-watch) for glucose determination. Glucagon (20 μg) is then administered by vein in zero time and the saline drip reattached. Ten minutes later venous blood is again withdrawn over a one-minute period of time. The anticipated normal rise in blood glucose in this period is 20 per cent $\pm$ 5 per cent of the fasting level. Less than this is considered to be a poor response to the glucagon. Many patients show little or no response whatsoever. These individuals seem to do well on Orinase and vice versa.

increases with such treatment, reverting to the original state of insensitivity on discontinuing the drug. The relationship of the drug's action to such change in sensitivity to glucagon suggests that the drug produces a block in the elaboration of intrinsic glucagon, (an absolute insufficiency of the hormone).

A rapid test for sensitivity to glucagon has been found to be useful in the selection of candidates suitable for this type of therapy.

CONCLUSIONS

1. The main hypoglycemic effects of the arylsulfonylureas are central in the liver.
2. The drugs have no detectable effect on the peripheral utilization of glucose.
3. They, accordingly, do not act as substitutes for insulin.

ACKNOWLEDGMENT

The authors desire to express their gratitude to Mrs. Agnes C. Dann, R.N., for her invaluable assistance in carrying through all of the blood glucose determinations involved in the study.

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New Experimental and Clinical Results with BZ-55 (Carbutamide) in Germany

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I am very glad to be here at the third BZ-55 Conference. In the short time available I can only make some general remarks about the development of the compound over the last six months.

We no longer receive the detailed reports as in the early trials. There are about 250,000 to 300,000 patients under treatment today. We receive only summarizing reports and in general more emphasis is put on negative observations. The following three points require particular attention: There is the question of chronic toxicity; study of allergic reactions is necessary; the possibility of a diabetogenic action of the compound must be considered.

1. Regarding chronic toxicity, there is nothing new to report. In Germany we have continued the treatment of our first twenty patients since February 1954; they have taken their tablets every day for two and a half years with no evidence of loss of effectiveness. Under careful clinical control there has been no sign of toxicity. The same applies to the great number of cases which have been treated for periods of one-half to one and a half years, and I think we are all agreed that chronic toxicity is slight. There appears to be no danger in long-term treatment with the compound.

2. It is more difficult to arrive at definite conclusions about the quality and quantity of allergic reactions. There seem to be significant differences between various countries. We had in West Germany 1.8 per cent of allergic reactions, in which are included the mildest, like itching, and also the more severe reactions, like fever and leukopenia. We are under the impression that the percentage is lower today, but there are strange accumulations in certain clinics and at certain times. In France, allergy seems to be extremely rare in the first two months of the use of the compound. In Switzerland, the number of patients treated has been relatively small, but they report 2.5 per cent of allergic reactions. In only a limited number of cases have we had to discontinue treatment or to substitute another oral antidiabetic substance.

3. If stimulation of the β-cells with enhanced output of insulin is the physiological mechanism of BZ-55, it is possible that BZ-55 might cause diabetes. We have given particular attention to the possibility of development of insulin resistance or deterioration of the metabolism. Authorities like Dr. Constam in Switzerland have also considered this point, but with the same negative results that we observed. Recently we received a report from a smaller German clinic stating that about eight cases were changed over from insulin to BZ-55 with initial success. But in about three weeks an increase in blood sugar occurred. After returning to insulin treatment the need for insulin was greater than before for

a certain length of time. I think, however, that these cases represent failures rather than proof of a diabetogenic action, as was the opinion of the reporting physician. We have no other reports of the diabetogenic action and I know of no animal experiments in which diabetes was caused by the compound.

Experimental findings are about the same as reported at the conference in March. More observations are published that BZ-55 acts even when β-cells are no longer present, as in complete alloxan-diabetes and in the depancreatized dog. At least, there is an action of BZ-55 if it is combined with insulin. It is also significant that the partially depancreatized dog easily goes into hypoglycemic shock when treated with BZ-55. It is difficult to reconcile this finding with the exclusive theory of the β-cell stimulation. There must be a peripheral mechanism and we find in the diaphragm of the rat in vitro an increased uptake of glucose but not an increased storage of glycogen by BZ-55 in therapeutic concentrations. On the other hand, Dr. v. Holt at the Institute of Prof. Kühnau has demonstrated hyperplasia of the islets in animals after long treatment with large doses of BZ-55. This is in agreement with the findings of the investigators in the United States and Canada. We have repeated the studies on the α-cells, especially when using chemically related substances. In a general concept of antidiabetic action the α-cells still cannot be neglected.

Finally we agree with Dr. Best that there is more than one point of attack in the body. We have to consider a peripheral, a β-cell and perhaps an α-cell action of BZ-55.

Metabolic Effects of Arylsulfonylurea Compounds in Normal Subjects and in Diabetic Patients

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In an effort to define the mode of action of sulfonylurea compounds our initial interest was directed at studies which sought answer to the following questions: (1) Do the sulfonylurea compounds suppress the pituitary-adrenal system? (2) Do the sulfonylurea compounds antagonize the peripheral effects of adrenal steroids? (3) Do the sulfonylurea compounds block the hyperglycemic effect of adrenalin and glucagon? (4) Do the sulfonylurea compounds increase sensitivity to exogenous insulin?

With these questions in mind extensive metabolic balance studies and numerous individual testing procedures have been performed before, during and following administration of BZ-55 and Orinase in normal and diabetic subjects.

At the time of the Second Conference on Substance BZ-55 in March 1956, we presented data[1] which indicated that the sulfonylurea compounds: (1) do not suppress the pituitary-adrenal system, (2) do not antagonize the peripheral effects of adrenal corticoids, and (3) do not block the hyperglycemic effects of glucagon and adrenalin. No evidence of potentiation of insulin activity could be demonstrated. Thus the blood-sugar-lowering property of these compounds was thought to be via another mechanism.

Since March of 1956 further extensive studies have been performed before, during, and following administration of carbutamide and Orinase in normal men and in various types of diabetic subjects.[2]

In general, the results of these studies have been consistent with the earlier interpretations and therefore the details of these studies will not be presented at this time.

In a further effort to elucidate the mode of action of sulfonylurea compounds a group of acute experiments have been performed on *normal subjects* to determine the effects of administration of insulin, on the one hand, and of Orinase, on the other, upon blood levels of glucose, pyruvate and alpha-ketoglutarate. The results indicate that when hypoglycemia is produced by admin-

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istration of insulin, the early changes in levels of blood pyruvate are opposite in direction from those observed when similar degrees of hypoglycemia are produced by Orinase[3].

METHOD OF INVESTIGATION

Eight insulin and nine Orinase experiments were performed. All subjects were given intravenously .05 units of glucagon-free insulin per kg. of body weight. In an additional experiment one subject received 0.1 units per kg. intravenously. Six subjects were given 1 gm. of sodium Orinase intravenously and one subject received 1.5 gm. intravenously. In addition, two subjects received 6 gm. of Orinase orally as a single dose.

During the intravenous tests blood samples for determination of glucose, pyruvate, and alpha-ketoglutarate were obtained at ten to thirty minute intervals. Levels of pyruvate and alpha-ketoglutarate were determined on whole blood by the paper chromatographic method of Seligson and Shapiro by Drs. Hennes and Wajchenberg.

RESULTS

Intravenous administration of insulin caused a mean fall in blood sugar of 48 per cent.

Also shown was the effect of intravenous administration of insulin on blood levels of pyruvate. In seven of eight experiments an increase in the level of blood pyruvate was the earliest change associated with hypoglycemia. In no instance did the level of pyruvate decrease before or during the time that blood sugar was falling which occurred within the first thirty minutes.

The intravenous administration of sodium Orinase caused a mean fall in blood sugar of 40 per cent.

The same experiments showed the effect of intravenous administration of sodium Orinase on blood levels of pyruvate. In contrast to administration of insulin, following intravenous administration of sodium Orinase, a decrease in level of blood pyruvate was the earliest change in five of seven experiments. This fall in level of pyruvate preceded the fall in blood sugar in two subjects. In only one experiment was the earliest change in blood pyruvate a significant increase within the first thirty minutes. Rises in blood pyruvate occurred after the first thirty minutes in several cases while the blood sugar was rising again and after symptoms of hyperadrenalemia had occurred.

It was during the first thirty minutes following intravenous administration of insulin and sodium Orinase that the most significant differences in blood pyruvate occurred in response to the two compounds. To illustrate that the differences in response of blood pyruvate are not dependent on greater or more rapid decrease of blood sugar following administration of insulin, the following findings are presented.

In two experiments we observed practically identical decreases of blood sugar produced by insulin and sodium Orinase in subjects T.S. and D.M. Nevertheless, the changes in levels of blood pyruvate were entirely dissimilar during the first thirty minutes. There was a marked rise in level of pyruvate following injection of insulin and a marked fall following administration of Orinase.

In subject D.W. we obtained a somewhat greater decrease in blood sugar following administration of sodium Orinase than following administration of insulin. The response to insulin was associated with a definite increase in level of blood pyruvate, while the initial response to Orinase was associated with a definite decrease in level of blood pyruvate.

There was no definite pattern of change in blood levels of alpha-ketoglutarate following intravenous administration of either insulin or sodium Orinase.

Finally, we showed that following oral administration of 6 gm. of Orinase the blood levels of pyruvate and alpha-ketoglutarate decreased at one hour and increased at two hours in each of two subjects. In one subject J.L. the decreases in blood levels of both intermediary metabolites preceded the fall in level of blood sugar and appeared fifteen minutes before symptoms of hypoglycemia. In both experiments the later increases of blood levels of pyruvate and alpha-ketoglutarate followed prolonged symptoms of hyperadrenalinemia.

SUMMARY

1. The acute changes in blood pyruvate and alpha-ketoglutarate associated with administration of insulin and of Orinase to normal subjects have been determined and compared.

2. The earliest change consisted of an increase in level of blood pyruvate associated with insulin-induced hypoglycemia, in seven of eight experiments. In none was there a decrease. In contrast, in seven of nine experiments the earliest change was a decrease in level of blood pyruvate when hypoglycemia was induced by Orinase.

CONCLUSIONS

The acute hypoglycemia following administration of insulin is usually associated with production of pyruvate in excess of its removal. On the other hand, acute hypoglycemia following intravenous or oral administration of Orinase is usually associated with removal of pyruvate

in excess of its production. These differences suggest that the immediate hypoglycemia induced by administration of insulin, on the one hand, and of Orinase on the other, occurs via different mechanisms.

These results do not support the concept that the sulfonylurea compounds produce acute hypoglycemia by stimulating rapid release of endogenous insulin.

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Group Discussion

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): One question about Dr. Anderson's report. I just wonder whether one can compare the results obtained at your time interval of a few minutes with those obtained when the blood sugar is lowered maximally by this drug in two to four hours. I just wonder whether you should have done the same experiment two to four hours after giving the drug?

GEORGE E. ANDERSON, M.D., (*Brooklyn*): We have done that in animals. In other words we have carried out investigations not only hours but days later. In the latter instance there *is* a depression. The methodology of these acute experiments will be thoroughly expounded in a coming issue of the *Journal of Clinical Nutrition.*

THOMAS H. MCGAVACK, M.D., (*New York City*): Regarding Dr. Achelis' paper, on what dosage level or levels were his patients carried for two and a half years without signs of toxicity?

J. D. ACHELIS, PROF. DR. MED., (*Mannheim-Waldhof, Germany*): One to two tablets or 0.5 — 1 gm.

LAURANCE W. KINSELL, M.D., (*Oakland, California*): In terms of peripheral effects and mechanisms, as we have reported previously, studies have been carried out in which patients have been maintained on constant diet containing essentially only fat. The objective was to produce major hyperketonemia and then to determine the possibility of modifying the hyperketonemia with particular reference to its progression. In studies that we have previously done, such a diet in any patient, diabetic or nondiabetic, without other measures being used, will proceed to marked ketoacidosis, and has to be interrupted because of this. In two such patients the administration of therapeutic amounts of the sulfonamide, either BZ-55 or on short-term experiments Orinase, has resulted in apparent major modification of this progression in that patients could be maintained indefinitely although with significant hyperketonemia but without ketoacidosis. This raises the question as to mechanism since we could know the total carbohydrates being metabolized from the urinary nitrogen on the one hand, and on the basis of the known glycerol moiety of the fat administered on the other. The question arose whether we might be having an increased oxidation of acetate peripherally as a result of BZ-55 administration. Two studies have been done using tracer doses of C^{14} carboxyl labeled acetate and determining its rate of appearance in expired CO_2 under controlled conditions. Our expectation was that there might be an increased amount of the C^{14} appearing in a stated period of time. The reverse has been found to be true. That is as far as we have gone.

HOWARD F. ROOT, M.D., (*Boston*): I would also like to ask Prof. Achelis a question, having recently had experience with one or two patients seriously ill with jaundice and other evidences of liver damage, particularly the type described by Dr. Duff some years ago. Now that the series has become so large, there will of necessity be patients who will die of other causes who have been receiving sulfonamides. Do you know whether any such patients have been studied with particular reference to the well-known pathologic findings in sulfonamide intoxication; that is, will there be an attempt to analyze, or is there anywhere any chance of getting some results of that sort? Two or three hundred thousand cases ought to offer a good many opportunities.

DR. ACHELIS: We have never seen jaundice in connection with our use of BZ-55. There have been patients on the drug with obstruction of the bile ducts. But in our reports there has been no jaundice resulting from the treatment.

DR. ROOT: I misunderstood you, Dr. Achelis. I assume you are saying that there have been some cases who, while receiving sulfonamides, have had jaundice which have been dismissed as jaundice not due to the

drug. Is that right?

Dr. Achelis: Yes, that is right.

Dr. Root: Well, that is one thing I did not get clear in my mind.

Dr. Achelis: No jaundice as a result of the treatment.

Dr. Root: On what basis are you sure of that? Is that on pathologic evidence?

Dr. Achelis: It is on pathologic evidence, yes.

Dr. Root: Do you mean that there are autopsied cases who had jaundice and in whom the findings were completely explained by the pre-existing liver disease?

Dr. Achelis: Yes, that is correct, cancer or infection.

James B. Field, M.D., (*Bethesda, Maryland*): Could I ask Dr. Anderson a question by way of clarification? Did you say that you would not expect a patient who had a normal response to glucagon to respond to Orinase?

Dr. Anderson: You are quite right. In the group of patients that we have been studying the ones who show a normal response to glucagon to a small dose, 10 to 20 mg., do not respond to the Orinase in a clinical sense, just as people who show an excess or response to insulin or a perfectly normal response to insulin, do not respond to the sulfonylureas. We have used this to determine which patients we shall put on the sulfonylureas even though they are of the obese-adult type of diabetic.

Dr. Field: If that's the case, I wonder if you have an explanation for the fact that nondiabetic normals presumably respond in a normal fashion both to glucagon and to insulin.

Dr. Anderson: I must say that I don't have an explanation except that in the normal dog we used excessive doses of the drug and so you can't gauge on that. I am referring to the straight clinical cases that we tested. I am not too sure that that is true in the normal.

Dr. Field: Isn't it fairly well documented that normal humans when given this drug will have a hypoglycemic response?

Dr. Anderson: Not all. Occasionally there are subjects without a hypoglycemic response.

Stefan S. Fajans, M.D., (*Ann Arbor*): In relation to that, 3 gm. of BZ-55 in four divided doses at six-hour intervals given to normal individuals keeps the blood sugar consistently around 60 mg. per cent although the control blood sugars would be around 85 mg. per cent. So in the therapeutic dose of 3 gm. given in divided doses, an effect in normal individuals can certainly be demonstrated.

George F. Cahill, M.D., (*Boston*): Fall in blood pyruvate is the first definite biochemical differentiation, I believe. I asked Dr. Fajans whether he has measured blood lactate. The reason I ask that is because we know that the sulfonamides may be nonspecific inhibitors of many enzymes, e.g., glucose-6-phosphatase, cytochrome oxidase, etc. If by this mechanism the lactate dehydrogenase or some redox system is inhibited a fall in pyruvate might occur which means nothing in reference to metabolic pathways, unless a comparable fall in blood lactate was found.

Dr. Fajans: I am sorry we did not measure blood lactate. We realize that the fall in blood pyruvate could occur if pyruvates were removed simultaneously or independently with stimulation of insulin secretion. All we can say at the present time is that the difference in response of the blood pyruvate following the treatment with Orinase and insulin respectively does not support the concept that sulfonylureas work by causing stimulation of endogenous insulin secretion.

Dr. Field: We have some information that might bear on Dr. Cahill's question. We have done some studies similar to Dr. Fajans' comparing the effect of insulin and Orinase on pyruvate levels. Our studies have been somewhat different from Dr. Fajans'. We've done these in diabetic subjects and we've given our insulin subcutaneously rather than intravenously. The reason I didn't comment on this before is because our results haven't been as consistent as his in showing in each case or almost each case a definite difference of the effect of insulin and Orinase, but we have also measured lactate and we haven't found any reciprocal change in pyruvate and lactate which might explain the pyruvate fall. In other words, we haven't observed a rise in lactate acid concomitant with the pyruvate fall or vice versa.

But what I would like to know is: Did the lactate fall parallel to the pyruvate? I'm not looking for the reciprocal change. But, if lactate acid falls parallel to the pyruvate then we can say there has been a completely altered C_3 metabolism which would be exceedingly important.

Garfield G. Duncan, M.D., (*Philadelphia*): I would like to come back to these patients with jaundice. Maybe I didn't get it quite clear, but I wonder if the patients with jaundice continued to receive the sulfonamide preparation and overcame their hepatic disturbance, or whether the drug was withdrawn and this disturbance cleared up and if so, was the drug reinstituted without a recurrence of hepatic trouble?

Dr. Achelis: We had one clinic where they were

treating a case of cirrhosis with BZ-55 and there was no further damage of the liver. There was a bigger group of cases where it has been demonstrated at autopsy that there is no connection between the treatment and the jaundice since they had pathologic findings in the liver, such as cancer. In other cases, I know that the treatment was continued and the jaundice did not recur.

A. E. RENOLD, M.D., (*Boston*): Could I ask Dr. Achelis one more question? Is there some evidence which you know about which indicates that the carbutamide can have an effect in the absence of the beta cells of the pancreas? And also some action on the glucose uptake on the diaphragm? I just wonder whether you could elaborate a little on this point.

DR. ACHELIS: I think there is some action if the beta cells are not present, and if you combine the insulin with BZ-55, you will have lowering of the blood sugar in alloxan diabetes and in the depancreatized dogs. And, therefore, it is my feeling that the exclusive theory of the stimulation of the beta cells is not sufficient. Your second question was about the diaphragm. At first we observed no change in the glucose uptake by the diaphragm. We repeated this test and found an increase in glucose uptake but we have not shown an increased storage of glycogen. These investigations are completely negated using the diaphragm from an alloxanized rat. Therefore, it is my feeling that in this case there must be a combined effect of insulin and BZ-55.

HENRY T. RICKETTS, M.D., (*Chicago*): I want to clarify this point still further if I may. I'm not sure we're quite clear about it yet. Do you think that in the total absence of the pancreas or the beta cells the sulfonylurea drugs have any effect if insulin is not given?

DR. ACHELIS: No, they have no effect.

DR. ROOT: May I ask one more question? You spoke of an incidence of 1 to 2 per cent of skin rashes. Has the drug always been discontinued in the presence of skin rashes?

DR. ACHELIS: Not in all cases. In only about 50 per cent of the skin rashes is it necessary to discontinue treatment with the drug.

DR. ROOT: Skin rashes are a puzzle to us because we have seen a variety of skin lesions other than mere itching. I speak now about a variety of eruptions, elevations, small and large, and I mean very large. In your experience have these types subsided and have you been able to resume the use of the drug?

DR. ACHELIS: We have found this in some cases but not in all.

DR. STADIE: We have been very much interested in the effects of the different media on the effect of insulin upon glucose uptake of metabolism of glucose on the diaphragm. Dr. Achelis, you mentioned that you were able to demonstrate an effect when you changed the media. Would you oblige us by being a little more detailed?

DR. ACHELIS: I do not have the data with me. I think I can send it to you later.

Metabolic Effects of Carbutamide in Diabetes and Interrelations with Glucagon

A Preliminary Report

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To elucidate the mechanism of the hypoglycemic action of certain sulfonamide derivatives, studies were undertaken to determine: a) effects of carbutamide on organic and inorganic metabolism in selected diabetic patients; and b) metabolic interrelationships of carbutamide and glucagon. The following daily measurements

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were used as indices of organic and inorganic metabolism: fasting blood sugar, inorganic phosphorus and eosinophiles in blood, urinary excretion of sugar, reducing corticosteroids, nitrogen, inorganic phosphorus, sodium, potassium, water balances, and body weight. The daily metabolic patterns are determined before, during and following the administration of carbutamide and glucagon given either singly or together in various sequences over periods of several days.

Throughout the two to three week periods of study each patient lived quietly under controlled conditions in the Metabolism Ward. Each patient received a chemically constant diet of identical foods and food values, the same menu being served every day. Carbutamide was administered orally in divided doses with meals. Glucagon was administered intramuscularly at 7 a.m., 3 p.m. and 11 p.m.

Studies have been completed on three patients so far. The metabolic data are presented in the accompanying four figures.

RESULTS

Patient one. The metabolic studies performed on the first patient (figure 1) were reported in part at our last conference. She was a sixty-nine-year-old white woman of normal weight with stable diabetes of about four years' duration who had taken insulin (40 to 45 units daily) for a little more than a year before admission. In the hospital she received a mixed diet of 2,100 calories containing 88 gm. of protein, 113 gm. of fat and 183 gm. of carbohydrate. On 26 to 30 units of NPH insulin and 12 to 14 units of crystalline insulin daily (period one) the fasting blood sugars averaged 208 mg. per cent and the twenty-four-hour urines contained in the neighborhood of 2 gm. of sugar. Omitting the dose of crystalline insulin resulted in a slight rise in urinary sugar (3 to 5 gm.). In this period the patient was in positive nitrogen balance and in inorganic phosphorus equilibrium. The daily levels of inorganic phosphorus in plasma were normal. The urinary reducing corticoids (4.5 to 5.5 mg. per twenty-four hours) were in the normal range. With the discontinuance of insulin (period two) there was a slow rise of sugar concentrations to about 250 mg. in the blood and 20 to 30 gm. in the urine. The daily urinary excretion of nitrogen and phosphorus increased slightly, the nitrogen balance changing from positive to slightly negative and inorganic phosphorus balance from equilibrium to negative. The daily urine output also increased slightly, and parallel with this body weight fell slightly.

Eight days after insulin was discontinued carbutamide was started (period three), the patient receiving 2.5 gm. on the first day, 1.5 gm. on the second and 1.0 gm. on the third. However, in view of the poor response the dose was gradually increased to 2.5 gm. daily. On the latter dose the fasting blood sugar ranged between 170 and 200 mg. per cent and the glycosuria between 3 and 6 gm. in twenty-four hours. The urinary excretion of nitrogen and phosphorus tended to decrease slightly, the nitrogen balance returning to slightly positive and the inorganic phosphorus balance to equilibrium. The urinary output and excretion of reducing corticosteroids returned to the levels of period one. Body weight remained steady.

The intramuscular administration of glucagon, 6.0 mg. daily for six days (period four), in addition to the carbutamide resulted in a prompt rise in the fasting blood sugar up to 300 mg. per cent and glycosuria of about 20 gm. in twenty-four hours. No consistent changes were noted in nitrogen, phosphorus or water balances. The number of eosinophiles in the blood rose sharply to double the value of the previous periods and continued there for the remainder of the experiment. The excretion of corticosteroids, however, did not change.

On discontinuing glucagon (period six), the blood and urinary sugar concentrations returned promptly to the same values as during the carbutamide period (period four). The levels of plasma inorganic phosphorus rose; the urinary excretion of corticosteroids did not change; weight and water balance remained steady.

On discontinuing carbutamide the blood and urinary sugar values again rose gradually, approaching those of period one. The rise in sugar levels began about the time carbutamide disappeared from the blood; that is five to seven days after stopping the drug. Nitrogen and phosphorus balances showed no consistent changes. The plasma inorganic phosphorus, urinary excretion of reducing corticosteroids, body weight and water balance remained steady.

Patient two. The second patient we studied was a seventy-year-old thin white woman with stable diabetes mellitus which had been discovered five years before her admission to the Metabolism Ward. This patient had been treated only by dietary restriction.

On a weighed mixed diet of 2,020 calories containing 86 gm. of protein, 98 gm. of fat and 199 gm. of carbohydrate and no insulin (period one), the fasting blood sugars ranged between 216 and 249 mg., average 231 mg. The urinary sugar ranged between 7 and 22 gm. per day, average 12 gm. The patient was in

PATIENT J.B. - FEMALE - AGE 69 YRS. - STABLE DIABETES

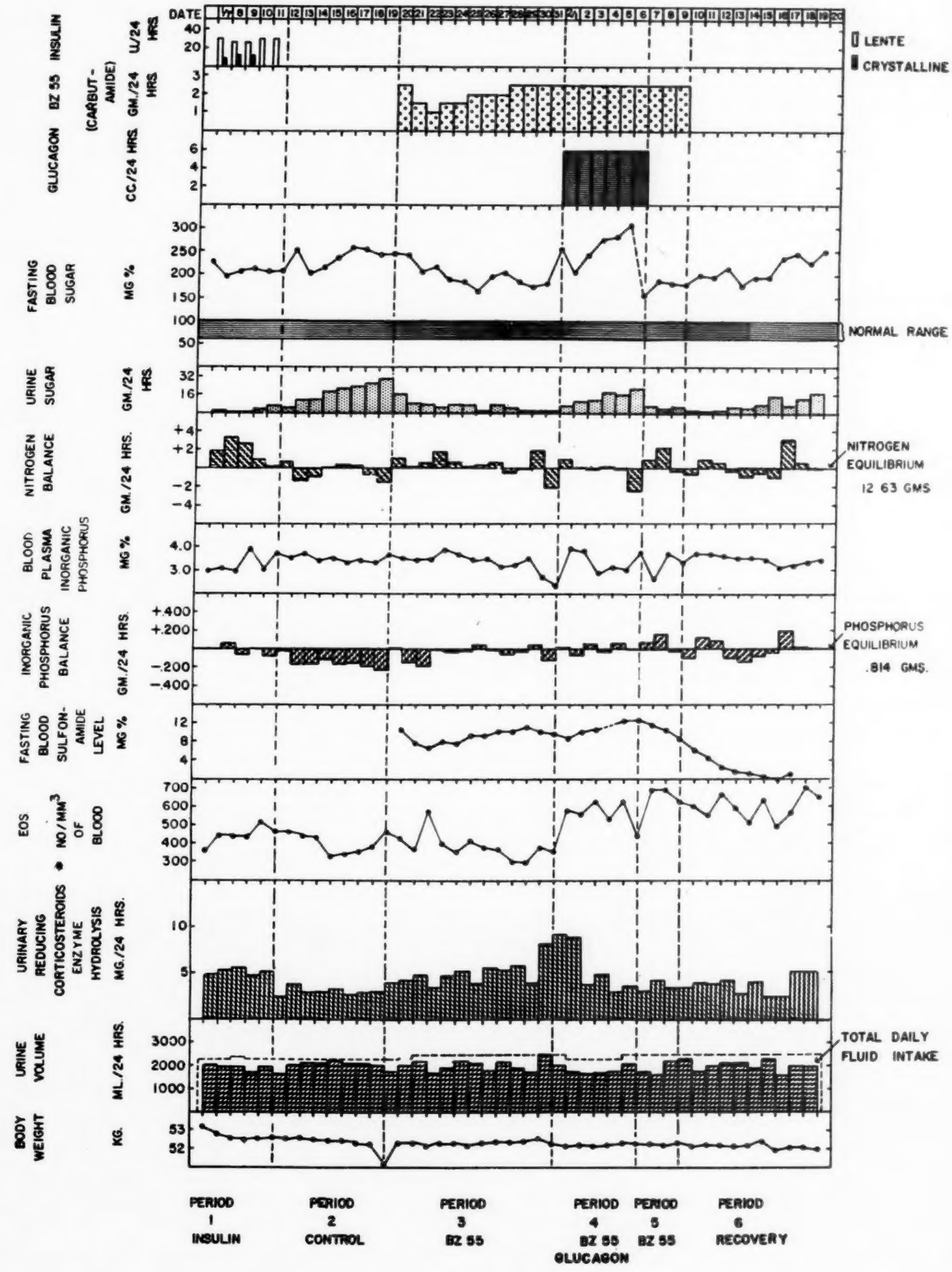

FIGURE 1

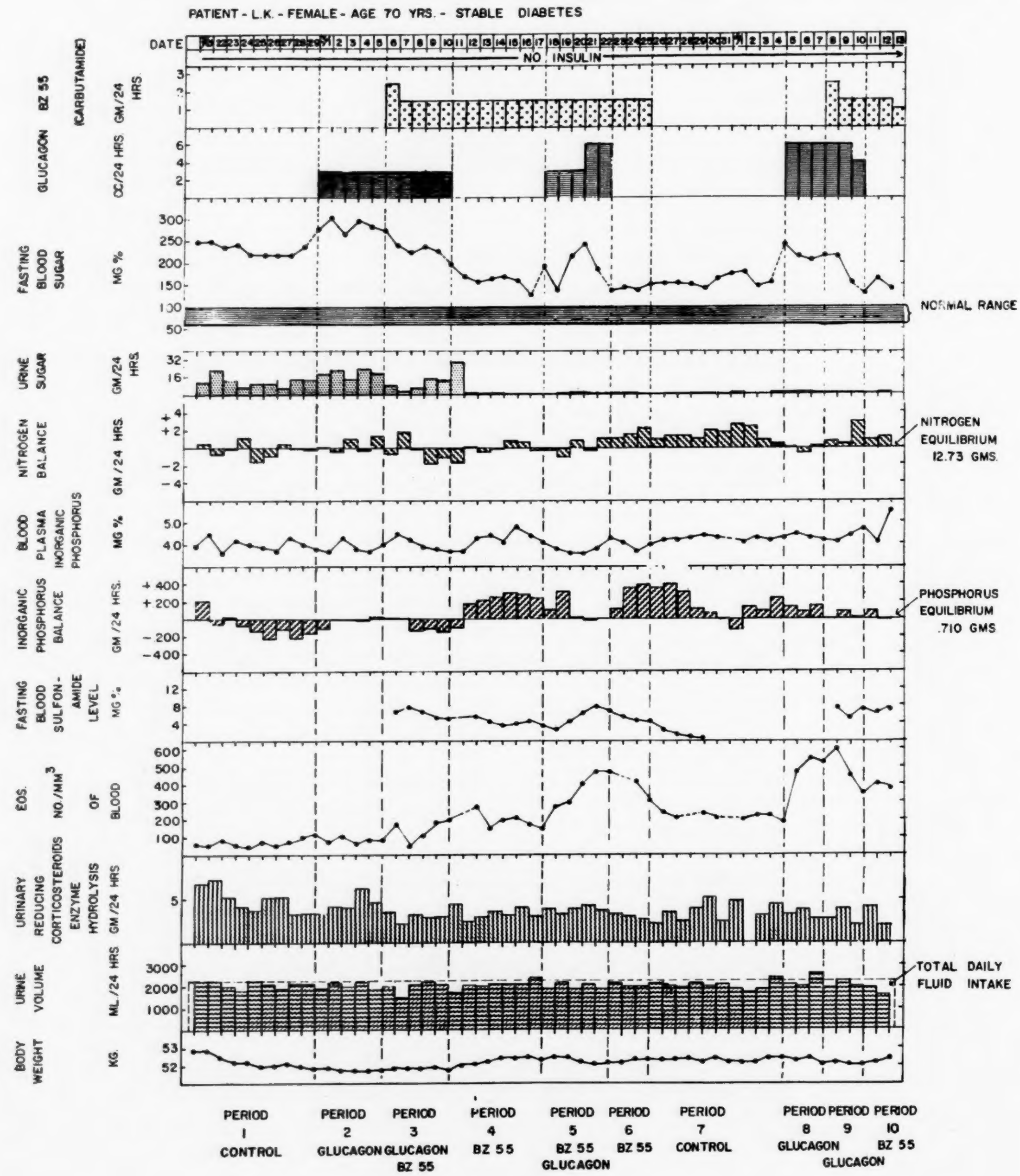

FIGURE 2

nitrogen equilibrium but in negative inorganic phosphorus balance. The daily inorganic phosphorus in serum determinations were in the normal range. The urinary excretion of reducing corticosteroids fell slightly toward the end of the period but the values remained within normal. This patient lost about 1.0 kg. of body weight in nine days.

The administration of 3 mg. of glucagon daily for

five days (period two) resulted in a prompt rise of blood and urinary sugar. The fasting blood sugars averaged 285 mg. per cent and the urinary sugars 22 gm. per twenty-four hours, representing an average increase of 54 mg. in blood sugar and 10 gm. in urinary sugar compared to the control during period one. Nitrogen and water balances did not change. There was a 20 per cent fall in average plasma inorganic phosphorus. The inorganic phosphorus balance changed from negative to equilibrium. Excretion of urinary corticosteroids increased slightly. Weight remained steady.

Carbutamide, 2.5 gm. the first day and 1.5 gm. daily thereafter (period three), appeared to counterbalance or cancel out the effects of glucagon. The average fasting blood and urinary sugar declined until they were similar to those of the control period. The average blood sugar was 232 mg. per cent, and the average urinary sugar, 10 gm. per twenty-four hours. Nitrogen and inorganic phosphorus balances tended to become negative. The number of eosinophiles in the blood rose slightly, and the urinary corticosteroids fell slightly, while her weight remained steady. On daily doses of 1.5 gm. carbutamide, the blood sulfonamide concentration varied between 4 and 8 mg. per cent.

After discontinuing the glucagon but continuing the carbutamide (period four), the average blood sugar fell from 232 mg. per cent to 157 mg. per cent and there was a virtual disappearance of glycosuria. The excretion of urinary nitrogen declined slightly. There was a rise in plasma inorganic phosphorus and a marked retention of inorganic phosphorus, resulting in an abrupt reversal of the phosphorus balance from negative to positive. A slight increase in body weight and number of eosinophiles in blood occurred; the urinary corticosteroids did not change.

On the thirteenth day of carbutamide therapy and the eighth day after stopping glucagon, it was again used at 3.0 mg. per day for three days (period five). The rise in fasting blood sugar averaged only 24 mg. per cent or slightly less than one-half the rise in blood sugar produced by the same dose of glucagon in period two. Increasing the dose of glucagon to 6.0 mg. daily for two days gave a blood sugar rise comparable to that of 3.0 mg. of glucagon in period two. However, neither the 3 nor the 6 mg. daily dose produced any glycosuria. The nitrogen balance did not change appreciably but the plasma inorganic phosphorus again fell. Urinary phosphorus increased with a resultant shift in balance from positive to equilibrium. The eosinophiles in the blood increased sharply particularly with the 6 mg. dose of glucagon. A slight rise in urinary corticosteroids occurred and body weight fell slightly.

During period six glucagon was discontinued but carbutamide was maintained. The nitrogen balance became positive. The plasma inorganic phosphorus again rose slightly and the inorganic phosphorus balance again became strongly positive. There was a sharp fall in eosinophiles and a slight fall in urinary corticosteroids.

The discontinuance of carbutamide for ten days (period seven) did not result in any appreciable rise in either blood or urinary sugar. The average fasting blood sugar of 157 mg. per cent was exactly equal to the average fasting blood sugar of the carbutamide period (period four). The patient remained in positive nitrogen balance. There was less retention of phosphorus but the balance remained positive. The increased excretion of phosphorus paralleled the fall in blood sulfonamide.

Ten days after stopping the carbutamide a third course of glucagon was started, this time at the 6.0 mg. daily dose level (period eight). Again there was a rise in fasting blood sugar averaging 73 mg. per cent higher than those of period seven, but glycosuria did not develop. Nitrogen balance changed from positive to equilibrium. The number of eosinophiles rose sharply as in period five, the second time glucagon was used. There were no changes in urinary corticoids or plasma inorganic phosphorus.

The addition of carbutamide (period nine) again counterbalanced the effects of glucagon on the blood sugar. Nitrogen balance changed from equilibrium to positive, and urinary corticoids again fell slightly as in period three.

The cessation of glucagon (period ten) resulted in a fall in blood sugar to levels equal to but not below the control levels of period seven, and again the eosinophile count fell.

Patient three. The third patient was a nineteen-year-old white girl with unstable diabetes of recent onset. Two months before admission to the Metabolism Ward she had been in the hospital with diabetic acidosis. Her history revealed that for two years she had had symptoms suggestive of diabetes mellitus but had not consulted a physician. During hospitalization the acidosis was controlled and her diabetes regulated by diet and insulin. Since discharge she had been fairly well controlled on diet and 80 units of Lente plus 20 units of crystalline insulin every morning before breakfast. On admission to the Metabolism Ward she was given a mixed, weighed diet of 2,134 calories containing 88 gm. of protein, 110 gm. of fat and 198 gm. of carbohydrate, and the same insulin dose that she had been receiving at home.

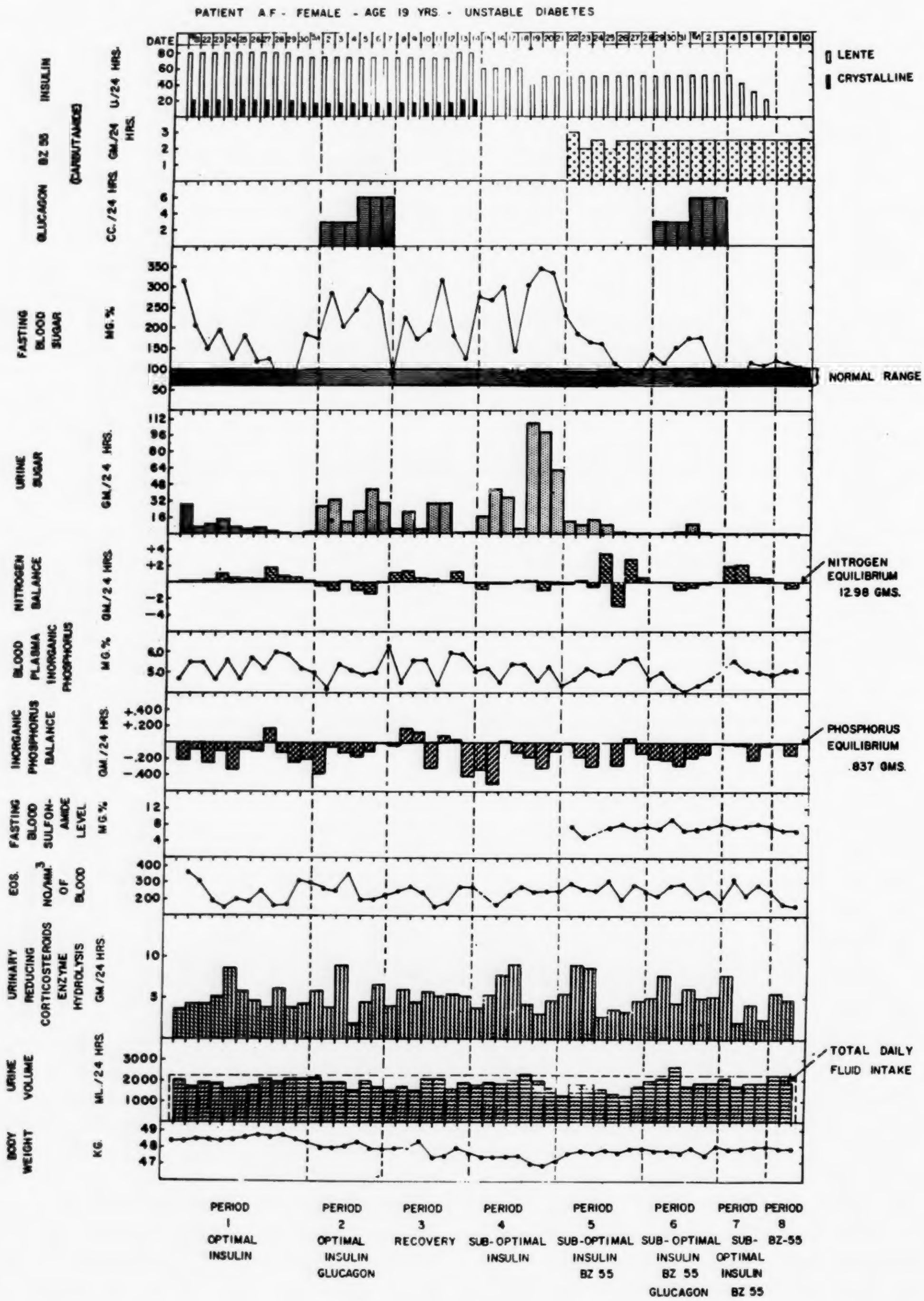
PATIENT A.F. - FEMALE - AGE 19 YRS - UNSTABLE DIABETES
DATE
INSULIN U/24 HRS.
LENTE
CRYSTALLINE
BZ 55 (CARBUTAMIDE) GM/24 HRS.
GLUCAGON CC./24 HRS.
FASTING BLOOD SUGAR MG.%
NORMAL RANGE
URINE SUGAR GM/24 HRS.
NITROGEN BALANCE GM/24 HRS.
NITROGEN EQUILIBRIUM 12.98 GMS.
BLOOD PLASMA INORGANIC PHOSPHORUS MG.%
INORGANIC PHOSPHORUS BALANCE GM./24 HRS.
PHOSPHORUS EQUILIBRIUM .837 GMS.
FASTING BLOOD SULFON-AMIDE LEVEL MG.%
EOS. NO./MM.3 OF BLOOD
URINARY REDUCING CORTICOSTEROIDS ENZYME HYDROLYSIS GM./24 HRS.
URINE VOLUME ML./24 HRS.
TOTAL DAILY FLUID INTAKE
BODY WEIGHT KG.
PERIOD 1 OPTIMAL INSULIN
PERIOD 2 OPTIMAL INSULIN GLUCAGON
PERIOD 3 RECOVERY
PERIOD 4 SUB-OPTIMAL INSULIN
PERIOD 5 SUB-OPTIMAL INSULIN BZ 55
PERIOD 6 SUB-OPTIMAL INSULIN BZ 55 GLUCAGON
PERIOD 7 SUB-OPTIMAL INSULIN BZ 55
PERIOD 8 BZ-55

FIGURE 3

On optimal insulin control (period one) the fasting blood sugars ranged from 60 to 200 mg. per cent and the urinary sugar from 1 to 15 gm. per day. Nitrogen balance was slightly positive. The plasma inorganic phosphorus ranged between 4.6 to 6.0 mg. per cent. The fluctuations in blood sugar and inorganic phosphorus were negatively correlated. The balance for inorganic phosphorus was negative. Urinary corticosteroid excretion was in the normal range. Body weight was steady.

The administration of 3 mg. of glucagon daily (period two) resulted in a prompt sharp rise in blood and urinary sugar. Increasing the dose of glucagon to 6 mg. daily produced still further rises in both blood and urinary sugar. During the glucagon period the fasting blood sugar varied between 180 and 300 mg. per cent and the urinary sugars between 12 and 45 gm. per day. Nitrogen balance changed from positive to negative, plasma inorganic phosphorus levels and body weight dropped slightly. Inorganic phosphate balance, water balance, blood eosinophiles and urinary corticosteroids did not change.

Discontinuance of glucagon (period three) resulted in a marked fall in blood sugar to normal but this was followed by irregular waves of hyperglycemia and glycosuria. Nitrogen balance again became positive and the plasma inorganic phosphorus levels began to rise although the individual daily values for phosphorus and nitrogen continued to be reciprocally related to the blood and urinary sugar concentrations. No change was noted in inorganic phosphate balance, blood eosinophiles, urinary corticosteroid excretion or water balance. Her weight continued to fall slightly.

Reduction in insulin dose from 100 units to between 40 and 60 units daily (period four) resulted in marked but irregular rises in blood and urinary sugar. On daily insulin, 40 to 50 units, the fasting blood sugar values were between 300 and 350 mg. and the urinary sugar between 60 and 110 gm. per day. Nitrogen balance reverted to slightly negative, plasma inorganic phosphorus tended to fall and urinary excretion of phosphorus tended to increase slightly. As in periods two and three her weight continued to fall slightly. There were no appreciable changes in eosinophile count or urinary corticosteroids.

During period five carbutamide was added while maintaining the insulin at a suboptimal dose of 50 units per day. With a daily dose of 2.5 gm. carbutamide, the blood sulfonamide concentration ranged between 5 and 10 mg. per cent. The fasting blood sugar gradually fell to normal and glycosuria disappeared altogether. The plasma inorganic phosphorus baseline tended to rise. No changes in blood eosinophiles, corticosteroid excretion or phosphate balance were noted. Body weight now tended to increase slightly and paralleling this urinary output decreased slightly.

In period six glucagon was administered along with suboptimal insulin and carbutamide. In marked contrast to the first glucagon period (period 2) the blood sugar rose only slightly but the urine remained free of sugar. Increasing the dose to 6.0 mg. per day for three days resulted in a further slight but variable rise in blood sugar. A mild glycosuria of 10 gm. was noted on only one day. During the period fasting blood sugars ranged between 100 and 170 mg. per cent. Although the blood and urinary sugar response was decidedly less than in period two, it is notable the same tendency for negative nitrogen balance and a fall in plasma inorganic phosphorus occurred. Inorganic phosphorus balance continued to be negative. No appreciable change in blood eosinophiles or urinary corticosteroids was seen. Body weight remained steady.

In period seven glucagon was discontinued and the dose of insulin progressively reduced from 50 to 20 units daily and finally discontinued altogether (period eight). The fasting blood sugar remained at nearly normal and the urine continued to be free of sugar. Nitrogen balance became positive, the plasma inorganic phosphorus increased and the phosphorus balance tended to become less negative. No changes in blood eosinophiles, urinary corticoids, water balance, or body weight were noted.

It is especially noteworthy that the fasting blood sugars during periods five through eight, while carbutamide was given, were decidedly more stable than those of the first four periods when the patient did not receive carbutamide. This is also reflected in the plasma phosphorus and to a lesser extent in the phosphorus balances.

Figure 4 demonstrates the daily potassium and sodium balances and the daily NA:K ratio in patient three for the eight periods described in figure 3. The most striking feature is the marked fluctuations in the daily urinary excretion of sodium whereas the excretion of potassium is relatively steady, thus resulting in an intense variation in the NA:K ratio. An inspection of periods one, two and three shows that glucagon had little or no effect on potassium or sodium balances. The administration of optimal insulin, periods one, two and three, was associated with potassium balances that varied between equilibrium and slightly positive, whereas suboptimal insulin, periods four to eight, was associated

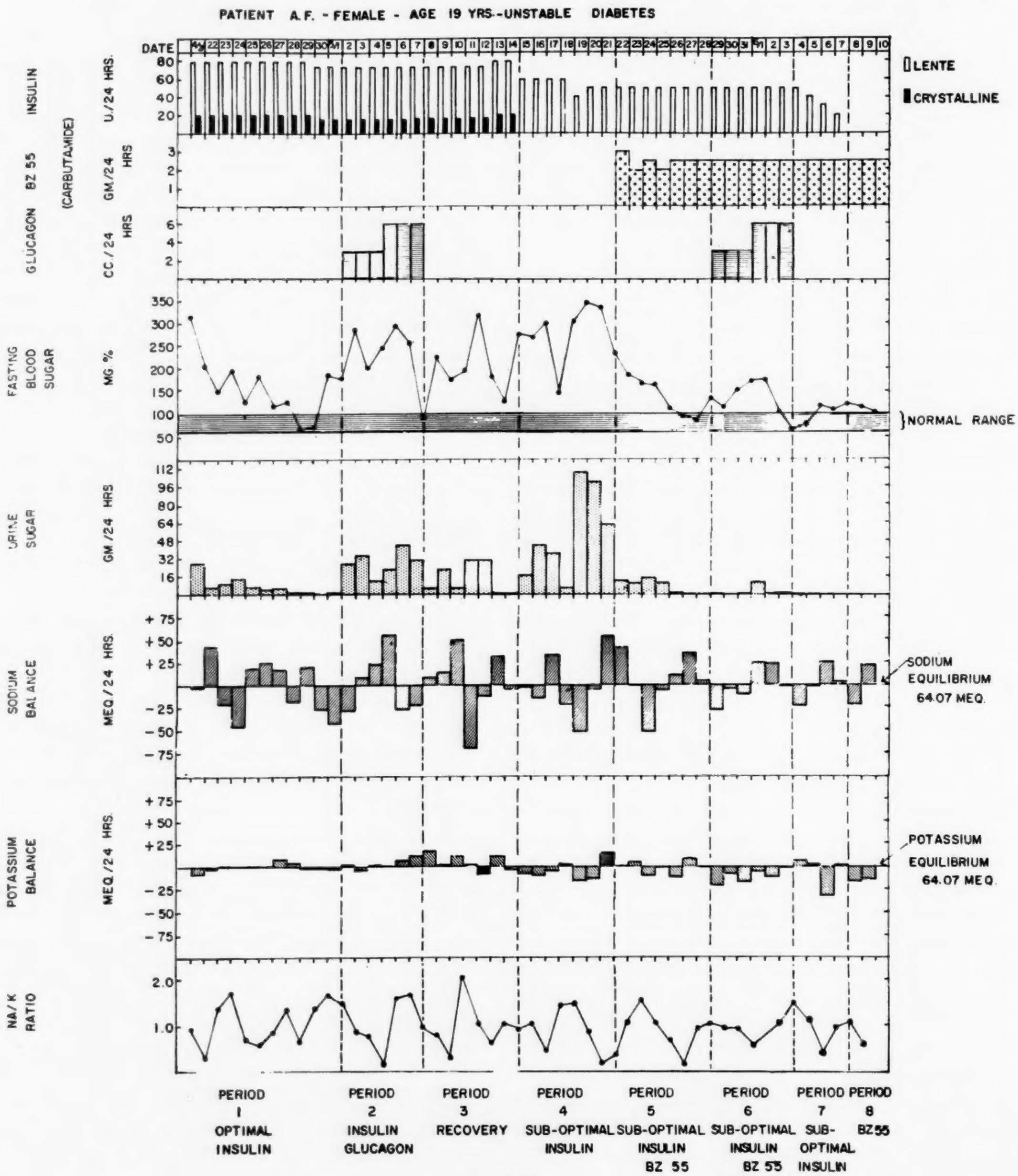

FIGURE 4

with a tendency for negative balances. Carbutamide did not appear to have any effect on potassium. However, during the administration of carbutamide the daily fluctuation in sodium balances tended to lessen. This was also reflected in the slightly steadier NA:K ratio.

SUMMARY

1. Detailed metabolic studies were made of three patients with diabetes mellitus who responded to carbutamide (BZ-55) by a lowering of blood and urinary sugar. Two were elderly women with stable diabetes

and the other a young girl with juvenile, unstable diabetes of recent onset. The hypoglycemic capacity of the drug varied among the patients studied. The most striking response was obtained in the young patient. In the other two patients the responses were mild to moderate.

2. The lowering of blood and urinary sugar by carbutamide was not associated with any consistent changes in the number of circulating eosinophiles or the urinary excretion of reducing corticosteroids. Carbutamide did not appear to have any direct effect on nitrogen, sodium, potassium or water balances, or body weight. In one patient the administration of carbutamide was associated with retention of inorganic phosphorus.

3. The repeated administration of glucagon for several days produced more or less sharp, sustained rises in blood and urinary sugar and a fall in serum inorganic phosphorus. In the unstable patient, the hyperglycemia and glycosuria induced by glucagon was associated with negative nitrogen balance. Effect of glucagon on nitrogen balance was also seen in lesser degree in one of the stable patients. These results are in agreement with other studies on glucagon in our laboratory and will be reported later.

4. The blood sugar lowering effect of carbutamide could be quantitatively antagonized or counterbalanced by the administration of glucagon. Conversely, the blood sugar raising effect of glucagon could be quantitatively antagonized or counterbalanced by the administration of carbutamide. The capacity of glucagon to raise the blood sugar appeared to be diminished to a greater or less degree in the presence of carbutamide in the two patients in whom this effect was investigated. Inhibition was noncompetitive. This inhibitory effect of carbutamide appeared to be directly proportional to its blood sugar lowering capacity. While carbutamide markedly decreased the hyperglycemic and glycosuric response to glucagon in the unstable patient, it did not proportionately diminish the effects of glucagon on nitrogen balance and serum inorganic phosphorus.

5. In the patient with unstable diabetes carbutamide produced not only a marked lowering but a stabilization of the blood sugar levels. This stabilizing effect was also reflected in the levels of plasma inorganic phosphorus, sodium balance and NA:K ratio.

CONCLUSION

These preliminary studies are consistent with the reported experimental observations that carbutamide reduces hepatic glucose output.

Studies of the Mechanism of Action of Sulfonylurea Derivatives

Martin G. Goldner, M.D., Bruno W. Volk, M.D., Shirley Weisenfeld, M.D., and Sydney S. Lazarus, M.D., New York

Since most of the presented experimental and clinical data are in print elsewhere,* only a brief summary will be given here. In addition, there appears in this issue a report of a joint project with Doctors Berson and Yalow of the Veterans Hospital, Bronx, New York, to which brief allusion was made during the symposium.

In the present study, no histologic changes of the pancreatic α cells of the rabbit were noted after oral or parenteral administration of hypoglycemic sulfonamides in various doses and over various periods of time. Degranulation of beta cells was observed after prolonged Orinase treatment.

No inhibitory effect of the action of exogenous glucagon by the hypoglycemic sulfonamides could be demonstrated in acute experiments in rabbits and in man, normal as well as diabetic. The height and the duration of the glucagon hyperglycemia showed no significant differences when induced with or without pretreatment with sulfonylureas.

Adrenalectomized dogs responded to the drug with unusually severe and prolonged hypoglycemia, while cortisone-pretreated rabbits responded like normal rabbits, but a single daily dose of the drugs was not sufficient to control the cortisone induced hyperglycemia and glycosuria over a twenty-four hour period.

From the Isaac Albert Research Institute and Department of Medicine, Jewish Chronic Disease Hospital, Brooklyn, New York.

* *Metabolism* 5:894-903, 1956.

No evidence of increased peripheral glucose utilization was found in man when the arteriovenous blood sugar difference was estimated after a glucose meal before and after sulfonylurea medication.

Clinically, generally satisfactory results were obtained in a group of twelve elderly diabetics treated over prolonged periods of time. It appeared that carbutamide was slightly more effective than tolbutamide when equal doses were employed. Only minor and transitory side effects of carbutamide were noted. In general, not more than 20 to 25 units of insulin per day could be replaced by the sulfonylurea derivatives.

The Effect of Sulfonylureas on the Rates of Metabolic Degradation of Insulin-I^{131} and Glucagon-I^{131} in Vivo and in Vitro

Solomon A. Berson, M.D., Rosalyn S. Yalow, Ph.D.,† Shirley Weisenfeld, M.D.,‡ Martin G. Goldner, M.D.,§ and Bruno W. Volk, M.D.,|| New York*

Recent studies on the hypoglycemic action of various sulfonylureas have not elucidated the mechanism by which their action is effected. However, the absent or diminished response of the blood sugar to these agents in the pancreatectomized or completely alloxan-diabetic animal has suggested that the hypoglycemic effect may be mediated through inhibition of insulin destruction or stimulation of insulin secretion. The possibility of an influence on glucagon secretion or glucagon degradation has also not been excluded. The present study was designed to evaluate the effect of the sulfonylureas on the rates of metabolic degradation of I^{131} labeled insulin and glucagon.**

METHODS

The preparation of the I^{131} labeled hormones, and the evaluation of alterations induced during preparation have been discussed in detail in previous communications.[1, 2, 3] A small moiety of the labeled hormones is frequently damaged, by irradiation or through other causes, and binds to serum proteins so that it leaves the blood stream less rapidly than the unaltered labeled hormone.[1, 2, 3] Since the fraction altered varies with each preparation, experimental and control studies were performed simultaneously with every lot of labeled hormone, with a single exception noted below. In vivo studies were performed in rabbits fasted for about eighteen hours; in vitro studies were carried out with rat liver homogenates. Equivalent doses of insulin–I^{131} or glucagon–I^{131} were administered intravenously to control rabbits and to sulfonylurea-treated rabbits and its disappearance from the blood stream was followed by an assay of radioactivity in washed trichloracetic acid precipitates of the plasma. The labeled hormones were given three to four hours after oral administration of 1-butyl, 3-p-aminobenzene sulfonylurea (BZ-55, U6987)* or 1-butyl, 3-p-tolylsulfonylurea (U-2043, D860, Orinase) or one to two and one-half hours following the intravenous administration of the sodium salt of Orinase (U7064). Both BZ-55 and Orinase† (orally and intravenously) were used in the insulin–I^{131} experiments but only Orinase sodium (intravenously) was employed in the glucagon I-^{131} studies.

The effect of Orinase sodium was tested on insulinase glucagonase and adrenocorticotropinase activity of rat liver homogenates at various concentrations of

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** We are indebted to Dr. O. K. Behrens and Dr. C. W. Pettinga of Eli Lilly and Company for a generous supply of crystalline regular insulin lot No. 535664 and to Dr. O. K. Behrens for a generous supply of crystalline glucagon.

* We are indebted to Dr. F. B. Peck, Sr., and Dr. W. R. Kirtley of Eli Lilly and Company for a generous supply of BZ-55.

† We are indebted to Dr. Cornelius J. O'Donovan of the Upjohn Company for a generous supply of Orinase.

Orinase, hormone and liver enzyme preparations. Slices of fresh rat liver were minced with two volumes of 0.067 M phosphate buffer, *p*H7.4, in a Virtis homogenizer. The homogenates were centrifuged at 2,000 g. for five minutes and the supernatant portion was employed either without further dilution or diluted again with 0.067 M phosphate buffer. Substrate mixtures containing labeled hormone together with measured amounts of unlabeled hormone were incubated at 37° C. with or without Orinase until temperature equilibrium was obtained. Liver homogenate preparations at 37° C. were then rapidly mixed with substrate solutions. At intervals thereafter, 0.2 ml. aliquots of the mixtures were pipetted into test tubes containing 5 ml. 10 per cent cold trichloracetic acid. Filtrates and precipitates were assayed separately in a 5 ml. capacity well scintillation counter with a sensitivity of 1.00 x 10^6 C./M./μc. I^{131} above a background of 200 C./M. Appropriate corrections were made, when necessary, for different volumes employed. All solutions of I^{131} labeled hormones were made up with added pooled human serum albumin to prevent absorption of significant amounts of the labeled hormones to the walls of glass containers.

Venous blood sugar concentrations were determined according to the method of Folin-Malmros.[4]

RESULTS

In vivo experiments. The disappearance of precipitable radioactivity from the plasma of control and sulfonylurea-treated rabbits given insulin–I^{131} is shown in figure 1. In all but two of the sulfonylurea-treated rabbits, the curves showed no distinct differences from those of the control rabbits. In one of the two exceptional cases, the rabbit died during the experiment in spite of the administration of intravenous glucose five minutes previously. In this case, the slow disappearance of precipitable I^{131} is conceivably related to a lowered circulation rate due to shock. In the other exceptional case, a control rabbit was unfortunately not studied with the same lot of insulin–I^{131}. It may be of interest that these two animals were the first to be studied in this investigation. Although many subsequent experiments were performed under apparently identical conditions, a slower than normal disappearance was never again observed. It has previously been demonstrated that the disappearance of precipitable radioactivity from the plasma is not

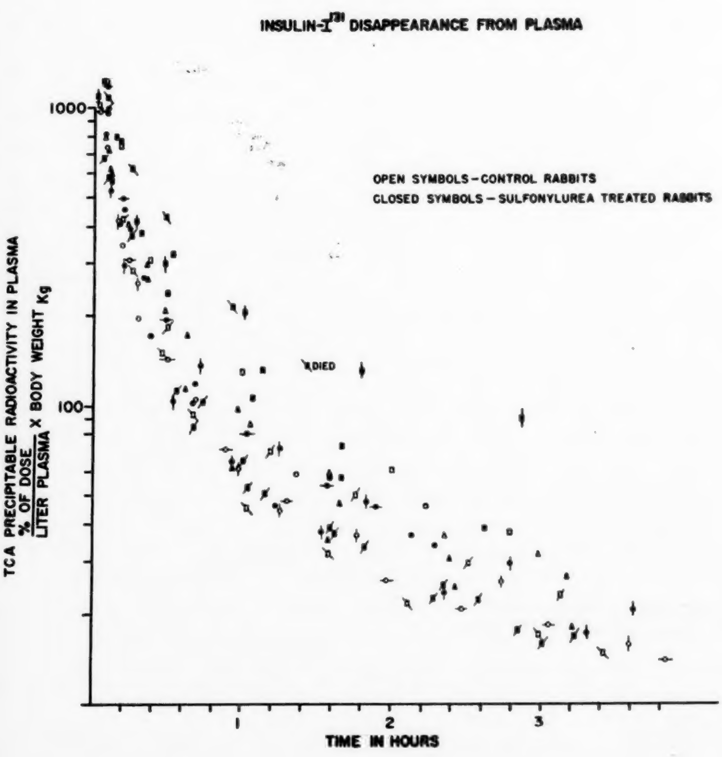


FIG. 1. Disappearance of precipitable radioactivity from plasma following intravenous administration of insulin-I^{131} to control and sulfonylurea-treated rabbits. The top two curves were obtained from rabbits given Orinase. The remainder were given either Orinase or BZ-55. See text for details. Like symbols indicate same lot of insulin-I^{131}.

due simply to extravascular distribution or organ concentration but is primarily a manifestation of metabolic degradation[1] as evidenced by the concomitant appearance of labeled monoiodotyrosine and iodide in the plasma.[3]

The disappearance of glucagon–I^{131} was not significantly different in Orinase-treated and control rabbits (figure 2a). The blood sugar responses to equal doses of glucagon (figure 2b) likewise showed no definite differences but it should be noted that the dose level of glucagon employed was apparently much above threshold level. In a previous study[3] significant hyperglycemia was observed with doses one-fourth as large.

In vitro experiments. In a variety of experiments in which different concentrations of liver, hormone and Orinase were employed, there was no detectable effect on liver insulinase (figure 3) or glucagonase (figure 4) activity at Orinase concentrations of 1 mg./ml. or less. At Orinase concentrations of 2.5 mg./ml. inhibition of liver insulinase appeared to be slight (figure 3) but became progressively more marked at higher concentrations. At 100 mg./ml. inhibition was virtually complete for both insulinase and glucagonase activities. At this concentration adrenocorticotropinase activity was also completely inhibited (figure 5).

DISCUSSION

The inactivation of insulin by tissue extracts was demonstrated by Mirsky and Broh-Kahn;[5] and Mirsky and associates[6] subsequently made a thorough study of the kinetics of the rat liver insulinase system. Mirsky[7] reported a noncompetitive inhibition of this system with the sulfonylureas and Mirsky, Diengott and Dolger[8] had suggested that this might be the mode of action by which the sulfonylurea drugs produce a hypoglycemic effect. However, Vaughan[9] working with insulin concentrations of 200 µg. per ml., failed to find any inhibition of insulinase activity in whole liver homogenates or partially purified insulinase systems with Orinase at concentrations of about 0.13 to 0.8 mg. per ml. or with BZ-55 at the latter concentration.

The present studies confirm not only that Orinase

GLUCAGON-I^{131} DISAPPEARANCE FROM PLASMA

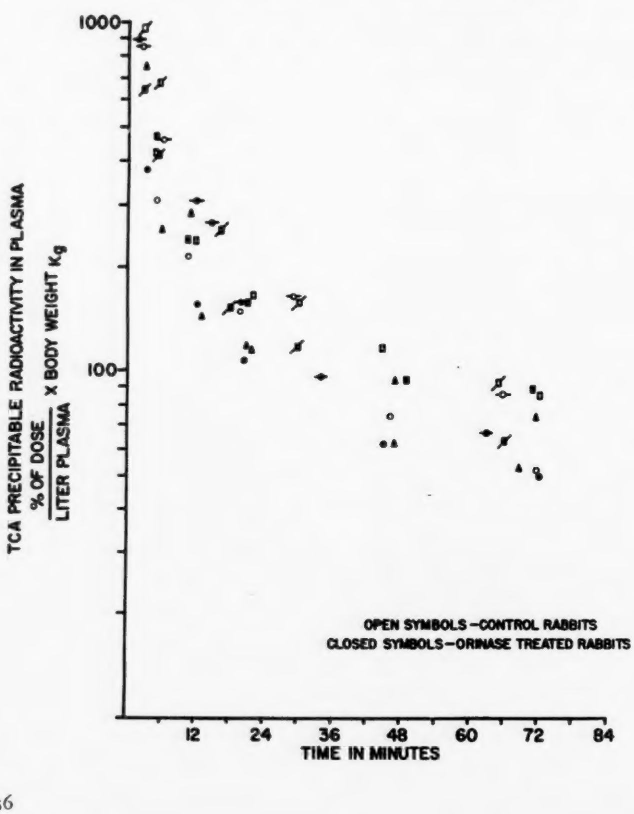


FIG. 2a. Disappearance of precipitable radioactivity from plasma following intravenous administration of glucagon-I^{131} to control and Orinase-treated rabbits. Like symbols indicate same lot of glucagon-I^{131}. Spiked symbols = 125 mg. Orinase per kg. body weight. Unspiked symbols = 250 mg. Orinase per kg. body weight.

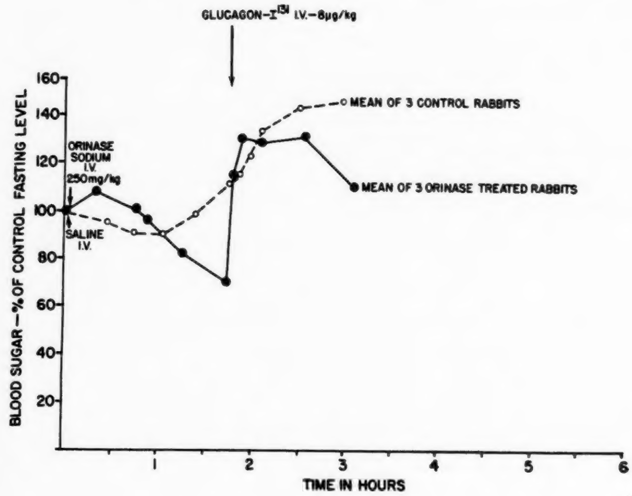
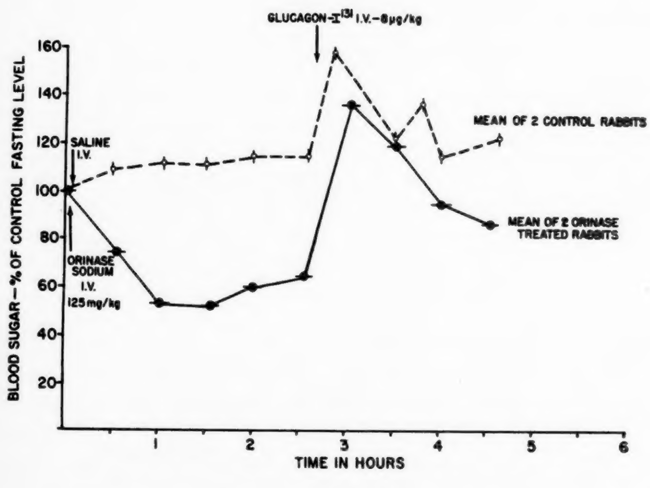


FIG. 2b. Blood sugar curves of same rabbits as in figure 2a.



in high concentrations is a strong inhibitor of liver insulinase but also indicate that at these concentrations it is also an effective inhibitor of liver glucagonase and adrenocorticotropinase activity as well. However, in agreement with Vaughan,[9] the observations reported here indicate that at concentrations which are sufficient to produce hypoglycemia, inhibition of insulinase or stimulation of glucagonase activity is not detectable. The blood levels of Orinase following oral administration of effective doses (25 to 100 mg. per kg. body weight) to dogs, generally do not exceed 0.1 to 0.25 mg. per ml. plasma,[10] but in the liver homogenate system it was not until concentrations of 2.5 mg. per ml. were reached that inhibition of insulin destruction was observed. The possibility that the sulfonylureas might be more concentrated in liver than in the plasma is not supported by the absence of concentration of S^{35} labeled Orinase reported by Baender and Scholz.[11] Even if such hepatic concentrations were obtained, the in vivo experiments reported here indicate that doses as high as 250 mg. per ml. given intravenously are generally insufficient to inhibit the destruction of insulin or glucagon. It is possible that the two exceptional cases in the insulin–I^{131} series represent instances in which there was such an inhibition, but the absence of inhibition in the majority of animals studied makes it seem unlikely that the

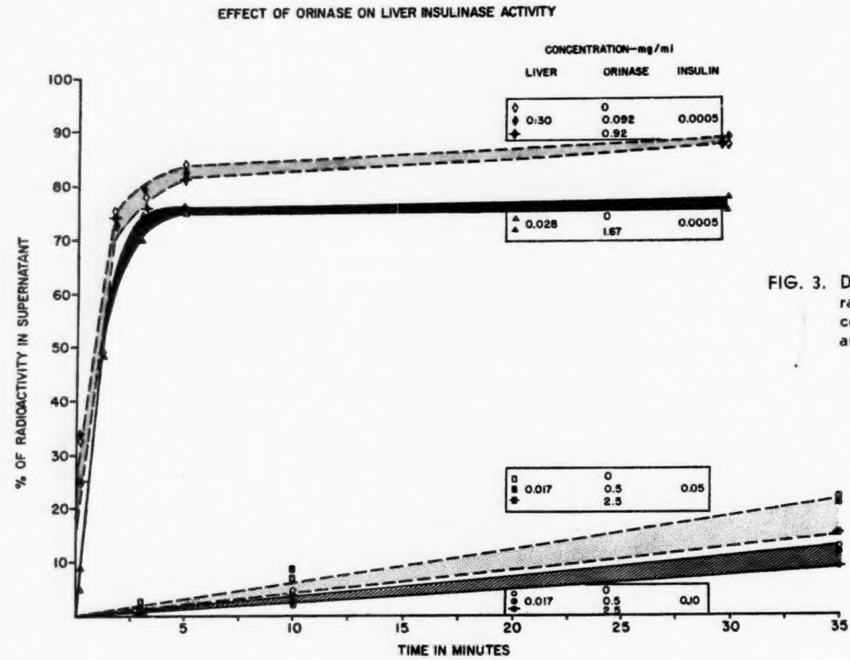


FIG. 3. Degradation of insulin-I[131] by rat liver homogenate at various concentrations of liver, insulin and Orinase.

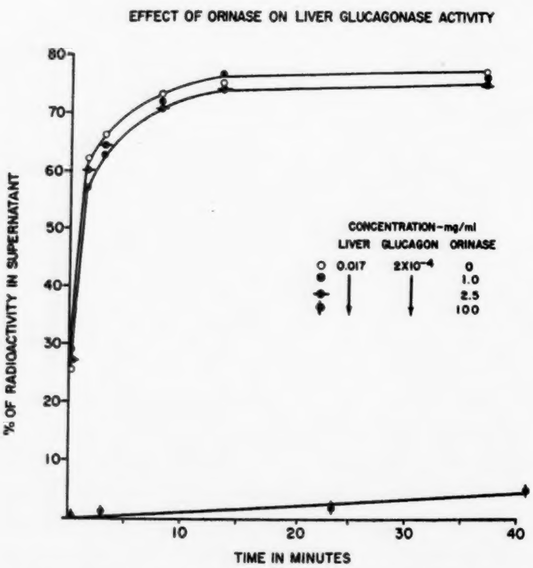


FIG. 4. Degradation of glucagon-I[131] by rat liver homogenate.

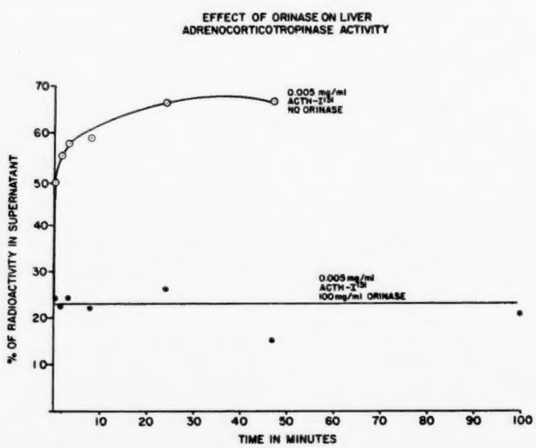


FIG. 5. Degradation of ACTH-I[131] by rat liver homogenate. Twenty per cent of this preparation of labeled hormone was not precipitable in control experiments without liver homogenate.

hypoglycemic action of the sulfonylureas is dependent on this mechanism.

The studies of Rall, Sutherland and Wosilait[12] have suggested that glucagon might act by stimulating some portion of the liver phosphokinase* system and

* This is the abbreviation employed by this group for dephosphophosphorylase kinase, a liver enzyme capable of reactivating liver phosphorylase after it has been inactivated (by removal of phosphate) by liver phosphorylase-inactivating enzyme. Both inactivating and reactivating enzymes have been identified and partly purified in their laboratory.

Vaughan's observations,[9] that in the presence of Orinase (0.8 mg. per ml.), glucagon fails to stimulate glucose formation in liver slices, led her to suggest that the effect of Orinase might be mediated through an inhibition of phosphokinase. The in vivo experiments of the present study do not support this action since the hyperglycemic response to glucagon was not any less marked in the sulfonylurea-treated rabbits than in the control group, although the possibility that a difference might be observed with smaller doses than were employed cannot be excluded.

Some suggestion of inhibition of glucose release by rabbit liver slices in the presence of BZ–55, 0.25 mg. per ml., was reported by Clarke and associates[13] although this effect was not observed by Vaughan[9] in the absence of glucagon or epinephrine even at higher concentrations of Orinase. Clarke and associates[13] observed a much more striking inhibition of cytochrome oxidase in liver slices at BZ-55 concentrations of 0.5 and 1.5 mg. per ml. Hawkins and coworkers[14] reported a significant depression of glucose-6-phosphatase activity, which Vaughan[9] had also suggested as an alternate explanation to the phosphokinase inhibition for the effects observed on release of glucose from the liver slice.

Both evidence and speculations about inhibition of a variety of hepatic enzymes are therefore not lacking from in vitro studies. Furthermore, the inhibiting effect on three apparently different hormone degrading enzyme systems observed in the present study questions the specificity of action of sulfonylurea on any enzyme system. Since it has been observed that insulin competes in both the glucagon and adrenocorticotropin systems and that glucagon competes in the insulin system,[15] the three hormone degrading activities may all be referable to a single hormonolytic enzyme. Nevertheless, the inhibitory effect of Orinase on this or these enzyme or enzymes, on cytochrome oxidase, and on one or more of the enzymes involved in the carbohydrate cycle, strongly suggests that the sulfonylureas may, in high concentrations, act as general enzymatic poisons. If so, the demonstration of any anti-enzyme effect in vitro is not sufficient to explain its hypoglycemic action unless this can be shown by experiments to result from the specific anti-enzyme effect in vivo at the lowest effective dose. By these criteria the action of sulfonylureas cannot be attributed to their anti-insulinase effect.

SUMMARY AND CONCLUSIONS

1. The administration of BZ–55 (orally) and Orinase (orally and intravenously) to rabbits, in doses sufficient to induce significant hypoglycemia, does not alter the rate of metabolic degradation of I^{131} labeled insulin or glucagon.

2. At Orinase concentrations of 1 mg. per ml. or less there is no detectable effect on rat liver insulinase or glucagonase activity. At significantly higher concentrations of Orinase there is inhibition of liver insulinase, glucagonase and adrenocorticotropinase.

3. It is concluded that the lowest concentrations of Orinase capable of inhibiting degradation of insulin and glucagon by rat liver homogenates are significantly in excess of those likely to be obtained by doses effective in producing a fall in blood sugar in the intact animal.

4. The hyperglycemic response to glucagon (8 μg. per kg. body weight) was not inhibited by the intravenous administration of Orinase.

ACKNOWLEDGMENTS

We are indebted to Mrs. Katherina Newerly, Mrs. Eunice Kratzer and Miss Marguerita Pascullo for the blood sugar determinations. We also wish to thank Mrs. Melanie Knopf and the staff photographers of the Medical Illustration Department of the Veterans Administration Hospital for the illustrations. The authors also acknowledge with thanks the secretarial assistance of Mrs. Frieda Steiner and Miss Eve Spelke, and the technical assistance of Mr. Carl Bacot and Mr. Herbert Lew.

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Intrapancreatic Infusions of the Sulfonylureas

John A. Colwell, M.D., and Arthur R. Colwell, Jr., M.D., Chicago

The influence of the sulfonylureas on the pancreas has been studied by introduction of these compounds into the pancreatic arterial circulation in twenty-seven experimental and control dogs. Small amounts of carbutamide or tolbutamide were injected into the pancreas, femoral vein or portal system. Injection was made into the pancreatico-duodenal artery through one of its branches, the right gastroepiploic artery, in such a manner that the pancreatic blood flow was not impaired. Peripheral venous blood glucose was estimated at intervals following infusion of amounts ranging from 3 to 71 mg. per kg. of body weight over periods of twenty or ninety minutes.

Hypoglycemic responses were obtained, the degree varying directly with the dose administered and the resulting blood sulfonamide level. Carbutamide in a dosage of 7 mg. per kg. of body weight given into the pancreas produced an average reduction in blood sugar of 20 per cent, even though the peripheral blood sulfonamide level (2 to 4 mg. per cent) was below the usual therapeutic range (10 to 15 mg. per cent). The same dose failed to lower the blood glucose when injected into the femoral vein in control animals subjected to sham pancreatic operations. Tolbutamide injected into the pancreatic artery in a dosage as low as 3 mg. per kg. of body weight also caused the blood sugar to fall. Hyperglycemia occurred when a blank solution lacking the compound was infused into the portal vein. The addition of a 7 mg. per kg. dose of carbutamide to this infusion produced no lowering of the blood sugar elevation.

Biopsies of the pancreas taken before and after infusion were examined by Professor W. Stanley Hartroft of Washington University Medical School. No significant histological changes in alpha or beta cells were reported. Insulin assays of pancreatic tissue performed by Dr. Carl Kuether of the Lilly Research Laboratories have not yet been completed.

These studies indicate that the sulfonylurea compounds cause hypoglycemia by some pancreatic mechanism, not as yet elucidated, and that the direct exposure of the liver to them causes no such effect.

From the Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

Summary of paper presented at the Third Conference on Carbutamide in Indianapolis on Sept. 13, 1956. Much of this material has been published in *Metabolism*.

Metabolic Studies with the Arylsulfonylureas

W. James Kuhl, Jr., M.D., Chicago*

During the past year clinical studies were undertaken to determine if the arylsulfonylureas would effectively control the stable diabetes mellitus of the nonobese patient after a sufficient period of dietary control alone had elapsed, if these compounds would potentiate the action of exogenous insulin in the patient with labile diabetes, and to determine the effect of these compounds upon blood glucose concentration when frequent small feedings of carbohydrate were given to the patient with diabetes mellitus. The plan of study, methods, and results have been published elsewhere.[1] They may be summarized as follows:

Ten patients with stable diabetes were transferred to a metabolic ward, given a weighed diet, and insulin discontinued or withheld. During a control observation period of at least fourteen days, five patients exhibited a progressive decrease in the degree of hyperglycemia and glycosuria although up to 25 units of insulin had been discontinued. Five patients who had stable hyperglycemia and glycosuria for a minimum of fourteen days were studied during chronic administration of the oral sulfonylureas. One of the latter five patients had only slight elevation of fasting blood glucose concentration with persistent glycosuria and showed only a minimal response. Following discontinuation of tolbutamide the diabetes has been controlled by diet alone for a period of five months. The remaining four patients exhibited a marked fall in fasting blood glucose levels and a decrease in daily glycosuria.

The administration of carbutamide to a patient with labile diabetes mellitus while continuing a reduced amount of insulin failed to reveal a potentiation of exogenous insulin.

Administration of a single large dose of arylsulfonylurea while feedings containing carbohydrate were continued every four hours, produced an equivocal decrease in blood glucose concentration in three individuals. A definite decrease in blood glucose values was noted after administration of a sulfonylurea in the fasting state.

Following these initial observations indicating an ability to lower fasting blood glucose concentration, an additional nine patients with stable diabetes were studied during chronic administration of the compounds. These patients were also transferred to a metabolic ward, insulin withheld or discontinued, and a measured diet was given. Fasting blood glucose concentration and twenty-four-hour glycosuria were determined twice per week. Qualitative urine tests were done four times per day. Three patients showed a progressive decrease in hyperglycemia and glycosuria, and were discharged on diet therapy alone. Six patients continued to have hyperglycemia during a control period of at least fourteen days. Five exhibited a prompt decrease in hyperglycemia to normal levels upon administration of the arylsulfonylureas. However, one of the five patients who showed this prompt decrease has not had a return of hyperglycemia or glycosuria following discontinuation of tolbutamide. One patient has had a partial lowering of fasting blood sugar and is now on an increased dosage of the sulfonylurea.

Determination of blood hemoglobin, white cell count and differential smear, urinalysis, serum alkaline phosphatase, BSP retention, two- and twenty-four-hour thyroidal radioiodine uptake has revealed no toxic reactions necessitating discontinuation of the compounds. One patient with relative lymphocytosis prior to therapy had no further reduction in neutrophils. One patient exhibited slight neutropenia which disappeared with continued therapy. One patient had nocturnal symptoms of hypoglycemia as an outpatient when he omitted his evening meal. One patient with BSP retention showed no improvement or deterioration of this liver function with carbutamide administration. One patient with generalized atherosclerosis had a second mild cerebral thrombosis, not associated with hypoglycemia, during therapy. In three instances there was a decrease in protein-bound iodine-^{131} and conversion ratio after tolbutamide or carbutamide. No evidence of thyroid enlargement during therapy has been noted.

From the Veterans Administration Research Hospital and the Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

This work was aided by grants from Eli Lilly and Company and The Upjohn Company.

* Assistant Professor of Medicine, Northwestern University Medical School and Staff Physician, Veterans Administration Research Hospital, Chicago.

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Group Discussion

GEORGE E. ANDERSON, M.D., (*Brooklyn*): We observed a dog for seventy-two days on 250 mg. per kg. per day. There were no observable changes whatsoever in the islet cells, certainly none in the alpha cells. This corresponds with Dr. Goldner's findings.

A. E. RENOLD, M.D., (*Boston*): I was interested in Dr. Izzo's observations on prolonged glucagon administration. It was not quite clear when the last dose of glucagon was administered before the fasting blood sugar was done. I also wanted to ask whether or not you have given glucagon over prolonged periods of time as in studies on normal subjects. It seems to me perhaps a little questionable whether one can interpret rises in blood sugar as meaning exacerbation of the diabetic state when the glucosuria did not increase. Perhaps the glucosuria is a better indication of over-all diabetic control than just one blood sugar value after giving an agent which affects blood sugar per se.

JOSEPH L. IZZO, M.D., (*Rochester, New York*): The glucagon was given at 7 o'clock, and the blood sugar was taken at 8:30. The administration of 6 mg. of glucagon daily caused a change in blood sugar from 100-150 mg. to about 300 mg. The most interesting feature is that this person then instead of having little or no glucose output increased it to 60 or 80 gm. per day. In addition there was a change in nitrogen balance and a fall in plasma inorganic phosphorus. But the most significant finding is this consistent effect on nitrogen balance. Whether this is due to glucagon itself or to some impurities in the glucagon, I can't say at the present time.

As for your other question about experiments on normals—we plan to use normals as controls. One of the difficulties in interpreting experiments on glucagon in normal individuals is that glucagon stimulates the pancreas to secrete insulin because of the increase of the blood sugar. We wanted to study the effect of the drug where the central secretion did not compensate for rises in blood sugar. We were surprised that glucagon could have a marked effect on blood and urine sugar if there is presumably no compensatory secretion of insulin.

LAURANCE W. KINSELL, M.D., (*Oakland, California*): As an assumption, those patients who respond to sulfonamide also make insulin. Also by assumption they don't do quite a normal job of it; otherwise they wouldn't be diabetic. The fact that they do respond and are not in the proteinuric-ketonemic group means that unquestionably they make perhaps quite appreciable amounts of insulin. Second, as I recall your figures, your increases, with perhaps one exception, were in urinary sugar predominantly in those patients who initially had high levels of blood sugar.

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): Dr. Izzo, what diet did you put those patients on?

DR. IZZO: Diets of approximately 2,000 calories with 90 gm. of protein, 200 gm. of carbohydrate, and about 100 gm. of fat.

Effects of Sulfonylurea Drugs in Hospitalized Diabetic Patients

Henry L. Wildberger, M.D.,* and Henry T. Ricketts, M.D.,† Chicago

The published literature available to us now (September, 1956) contains reports of at least 1,035 patients treated with the sulfonylurea compounds, and doubtless many times this number have actually received the drugs. As nearly as can be ascertained, all but thirty of these patients have been studied and evaluated by methods which are either incompletely described or, in our opinion, inadequate to permit valid conclusions as to the efficacy of the treatment. Unpublished reports presented at this symposium are not included in this survey.

Methods that are satisfactory for such studies include the use of patients with well-established diabetes of relatively remote onset, hospitalization (preferably on a metabolic ward), a sufficiently long fore-period to ensure a stabilized diabetic state, provision of a diet that maintains body weight, administration of the test substance when blood and urine sugars are fairly constant or rising, and the employment of changes in well-marked hyperglycemia and glycosuria rather than changes in the so-

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called insulin requirement as the criterion for judging the results of drug therapy.

The present study is concerned with the effects of carbutamide and tolbutamide in seven diabetic patients observed under conditions which as nearly as possible approximated those just described. Samples of blood for determination of glucose were obtained four times each day, and daily twenty-four-hour specimens of urine were collected for quantitative analysis. Dosage of the drugs was from 1.5 to 3 gm. daily, and blood sulfonamide levels in the cases in which they were determined were in the accepted therapeutic range of 15 to 20 mg. per 100 ml.

Results are shown in the accompanying table.

DISCUSSION

Of the seven patients studied, five would be expected to respond satisfactorily to drug therapy on the basis of earlier reports, i.e., they were obese, middle-aged or elderly individuals with mild to moderately severe diabetes of only a few years' duration. Of the five, two (cases 1 and 7) showed a partial response but insufficient for proper diabetic control without insulin; one (case 3) responded partially in the hospital but very well later on ambulatory management; one (case 5) had no response; and one (case 6) obtained a striking therapeutic result. Of the two patients in whom a favorable effect was not anticipated, one (case 4) was a juvenile diabetic whose condition was unchanged by the drugs and the other (case 2) was a patient with diabetes of sixteen years' duration which could not be properly controlled with drug therapy alone.

Hypoglycemia did not occur in any of these cases with drug alone. With two exceptions, no evidence of toxicity was found by the usual clinical and laboratory tests. In one patient (case 2) the white blood count was 5,750 per cu. mm. with 46 per cent granulocytes prior to treatment. After treatment with tolbutamide followed by carbutamide, the white blood count was 3,300 per cu. mm. with 29 per cent granulocytes. In a second patient under similar circumstances, the initial white blood count was 12,000 per cu. mm. A fall to 4,300 per cu. mm. was noted on treatment with carbutamide.

CONCLUSIONS

1. Carbutamide and tolbutamide have hypoglycemic activity in some but not all diabetic patients.

Summary of results in hospitalized patients

Case, age, sex, nutritional state	Duration diabetes (years)	Duration insulin therapy (years) usual dose (units)	Duration hospital observ. (days)	Insulin dose while on drugs (units)	Average blood sugar level (mg./100 cc.) Average daily urinary glucose (gm.) Control period	Drug period	Recovery period	Drug period	Remarks
(1), 36, F, obese	1	1 40	63	10	200 44	C* 160 12	200 34	T* 187 10	
(2), 70, F, obese	16	10-12 30-35	57	0	262 32	T 237 15	286 43	C 251 26	
(3), 68, M, obese	0.25	0	37	0	271 50	T 260 13	–	–	Urine became sugar-free and blood sugars normal on drug at home.
(4), 24, M, nor. wt.	5	4 45	54	45	199 34	C 241 58	–	T 206 50	On anti-convulsant drugs for mild epilepsy. No potentiation of barbiturates noted.
(5), 59, F, obese	1	0.6 25	17	0	149 0	C 160 0	–	–	Total adrenalectomy 1 year previously. Maintained on 50 mg. cortisone daily.
(6), 60, F, obese	4	4 50	90	0	365 61	T 120-140 0	280-300 9	C 120-140 0	
(7), 47, F, obese	1	2 days 10	56	0	311 50	T 282 18	287 23	–	On carbutamide at home. Urine is essentially sugar-free. Postprandial blood sugars, 198 and 172.

*C=carbutamide.
*T=tolbutamide.

2. Even among patients who, on the basis of previous reports, might be expected to respond favorably, the drugs are in some cases incapable of controlling diabetes adequately without injected insulin.

3. The results of clinical studies under carefully controlled conditions suggest that the usefulness of these preparations may be considerably more limited than was originally expected.

4. The occurrence of leukopenia in two patients of this group treated with carbutamide after receiving tolbutamide calls attention to the toxic potential of these drugs.

Observations in Diabetic Subjects Treated with Sulfonylurea Compounds

Stanley J. Talpers, M.D.,[*] Thomas Stanford Splitter, M.D.,[†] Roger Friskey, M.D.,[‡] Frederick Brown, M.D.,[§] Laurance W. Kinsell, M.D.,[‖] Oakland, California

This report summarizes some of the experience of members of the Institute for Metabolic Research with the oral hypoglycemic agents during the period January-October, 1956.

OUTPATIENT STUDIES

An initial group of twenty-eight patients was selected in March and April of 1956. None of these was an "ideal" diabetic in terms of precise adherence to a diet, etc. These patients, for the most part, were obese, middle-aged or elderly, nonketonuric, diabetic patients whose insulin dose varied from zero to forty units. As shown in figure 1, the average fasting blood sugar values were unchanged after insulin withdrawal. (Some of these data have been reported by Splitter et al.)[1] In only one patient was it necessary to resume insulin because of ketonuria and rising blood sugar. All but four of the patients in this group showed significant reduction in blood sugar values while on carbutamide or tolbutamide. A mild granulocytopenia in one patient while under carbutamide therapy was the only side effect noted. Hypoglycemic episodes were not encountered, nor have patients become unresponsive to the drug.

The second group of thirty-two patients included five "unstable diabetics." These five and three others have failed to respond to sulfonylurea therapy (figure 2). Withdrawal of insulin for four weeks prior to oral treatment produced little change in average fasting

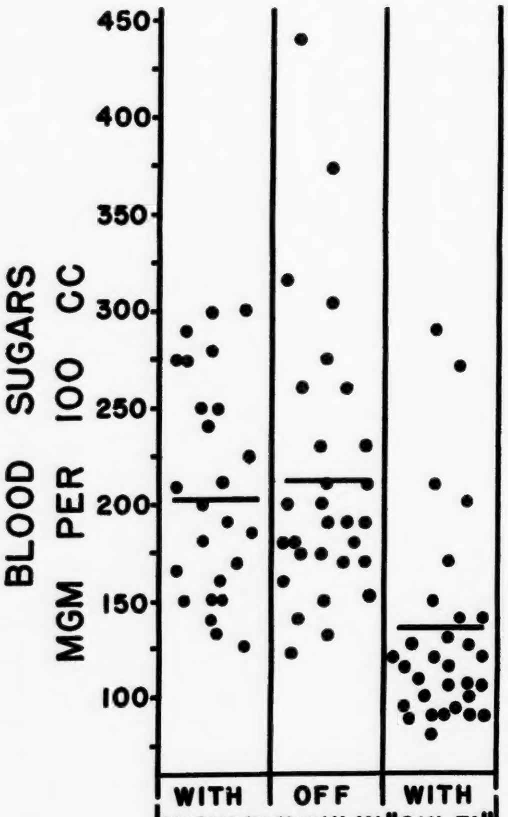

FIG. 1. Fasting blood sugar determination in twenty-eight ambulatory diabetics (a) during treatment with insulin, (b) following cessation of insulin therapy and (c) during sulfonylurea therapy.

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§ Research Fellow, Institute for Metabolic Research.

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These studies have been supported in part by grants-in-aid from Eli Lilly and Company and from The Upjohn Company.

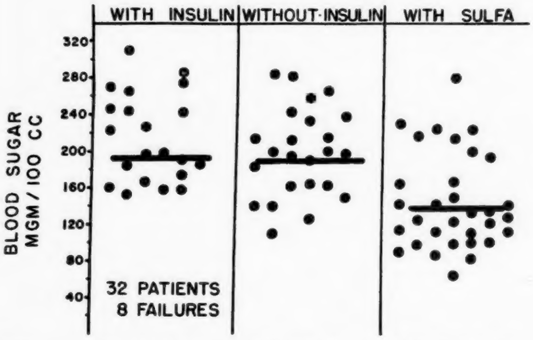

FIG. 2. Fasting blood sugar determination in thirty-two ambulatory diabetics with and without insulin and with sulfonylurea.

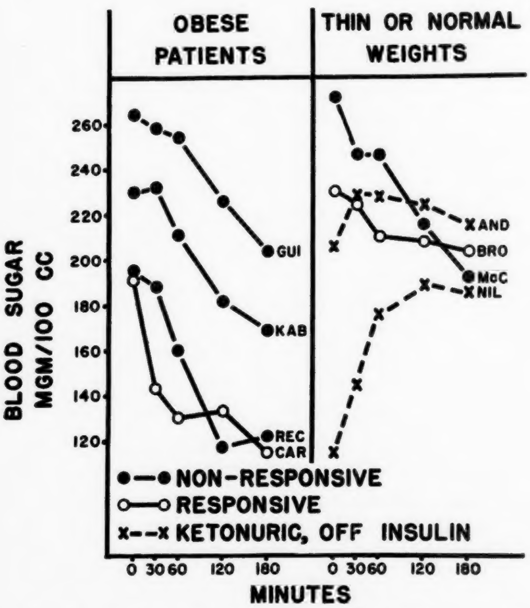

FIG. 3. Representative responses (in fasting state) to 2 gm. of sodium Orinase administered intravenously in eight diabetic patients.

blood sugar values. Dosage for patients in this group varied from 0.5 to 3.0 gm. per day, usually in one morning dose.

Side effects in this group have been minimal. There have been no rashes or drug fevers. One patient has noted desquamation of one palm.

In both groups we have been puzzled by the occasional nonketonuric diabetic who fails to respond to sulfonylurea treatment. For that reason, we have attempted to "screen" patients by administering sodium tolbutamide intravenously. A three-hour test, using 2 gm. of sodium

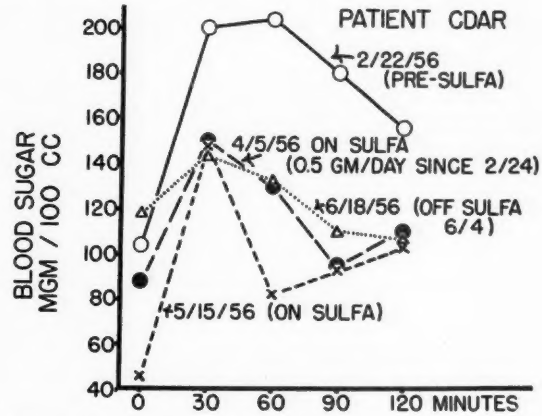

FIG. 4. Normalization of glucose tolerance in response to sulfonylurea therapy in an early juvenile diabetic.

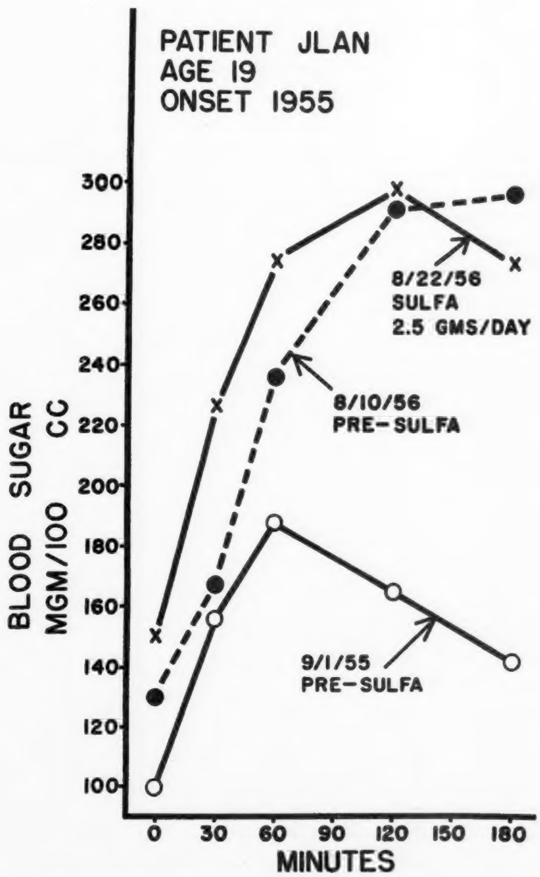

FIG. 5. Lack of response to sulfonylurea in a juvenile diabetic (duration of the disease more than one year).

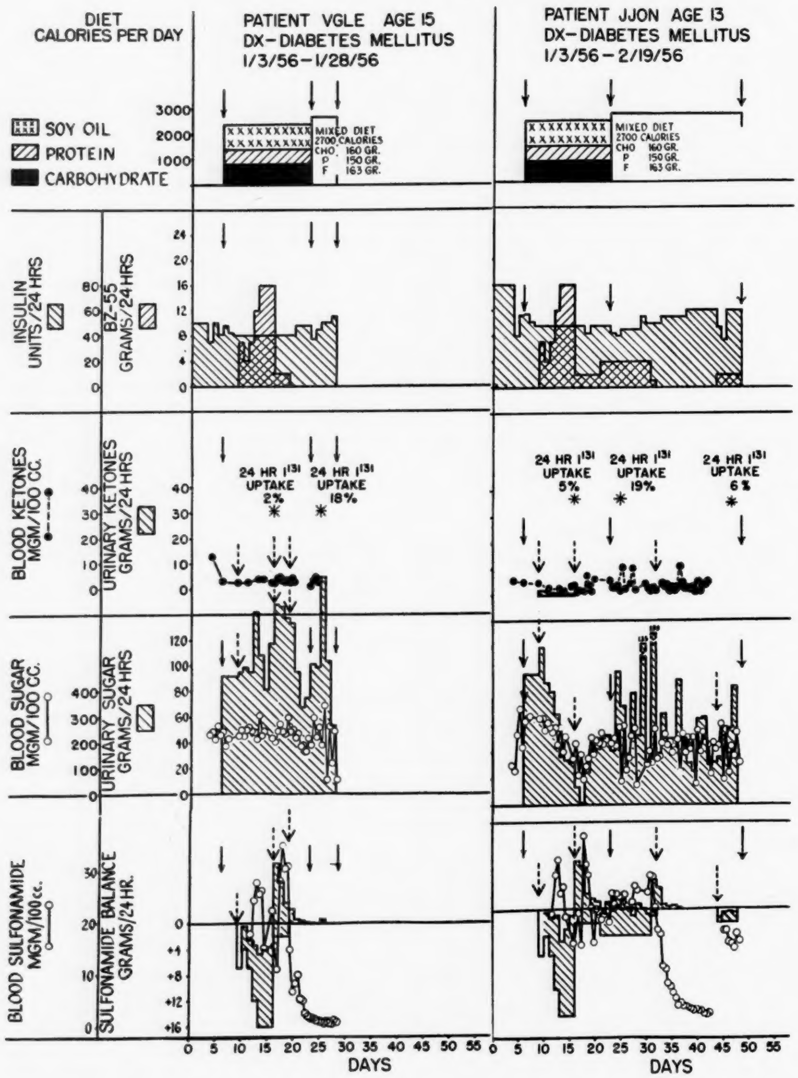

FIG. 6. Effects of massive carbutamide administration to unstable juvenile diabetics, age 13 and 15.

tolbutamide, has been performed on twenty patients. Figure 3 shows some representative results. Except for "ketonuric diabetics" it is apparent that the three-hour intravenous test will not separate responsive from nonresponsive patients.

The juvenile diabetic is reported to be usually unresponsive to sulfonylurea therapy. In figure 4 are shown glucose tolerance curves in a child with "preclinical" diabetes. Glycosuria was noted in a routine urinalysis in February 1955. Glucose tolerance at that time was grossly abnormal. He was placed on a high protein, high fat diet, and observed until February 1956. Glucose tolerance at this time was still grossly abnormal. Response to carbutamide is shown. In June 1956, it was necessary to discontinue therapy temporarily because of the appearance of hypoglycemic symptoms. He is presently satisfactorily maintained on 250 mg. every other day.

In contrast to this patient, figure 5 shows glucose tolerance curves of a nineteen-year-old college student with progressive diabetes of one year's duration in whom a dose of 2.5 gm. of carbutamide per day was ineffective.

METABOLIC WARD STUDIES

Two juvenile diabetics (previously reported[2]) age 13 and 15, were studied on the metabolic ward simultaneously. The degree of severity of their diabetes was similar and their programs were identical in a quantitative chemical sense. Insulin was gradually withdrawn until both were spilling approximately 100 gm. of urinary sugar per day. Carbutamide was then added in doses ultimately reaching 16 gm. per day. Figure 6 shows the results in both boys. The first was responsive to the large dosage used. The second showed increased glycosuria without a concomitant increase in blood sugar, suggesting a renal effect. During this study it was noted that the responsive youngster was excreting the carbutamide mostly in the acetylated form, in contrast to the nonresponsive boy in whom the major portion of the drug was present in the free form. Further studies which we hope will throw light on this variation are in progress.

SUMMARY

We have reported some of our experiences with two oral sulfonylurea compounds in sixty ambulatory diabetics. The drugs were effective in approximately 75 to 80 per cent of the cases and side effects were not a serious problem. Initial experiences with an intravenous sodium tolbutamide response test suggest that it is of little value in predicting which patients will be responsive.

The diabetic glucose tolerance test of one nine-year-old boy was changed to normal by small doses of carbutamide. In another juvenile there was no change in the curve while on the drug. Balance studies in two hospitalized diabetics were paradoxical in that, while on identical programs one youth was responsive, the other, unresponsive.

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Sudden Death in a Diabetic Subject During Treatment with BZ-55 (Carbutamide)

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Sulfonylurea derivatives have been recently introduced as antidiabetic agents.[1-4] The drugs are receiving wide clinical trial, and to date reports of toxicity have been limited to several cases of drug rashes[3] and two cases of neutropenia.[5, 6] We recently observed a patient who died suddenly while on a sulfonylurea compound, (N_1-sulfanilyl-N_2-n-butyl-carbamide, BZ-55, carbutamide). Autopsy disclosed lesions similar to those reported previously in association with drug toxicity.[7-10] In view of the interest in these new agents the following case is reported in detail.

CASE REPORT

F.W., Number 5337, a forty-eight-year-old colored female domestic, was admitted to the Clinical Center on Feb. 24, 1956, for study of periodontal disease and diabetes. Diabetes had been discovered in 1954 and had been controlled with 10 to 15 units of insulin and a 1,200 calorie diet for eight months. At that time, one year prior to admission, she discontinued insulin because "injections were raising knots in my skin." Thereafter, except for some pruritus which she associated with dietary excess, the patient had no symptoms attributable to diabetes. There was a positive family history for diabetes.

Past history and review of symptoms were unremarkable except for history of moderate to excessive intake of alcohol.

Physical examination on admission revealed a moderately obese colored female in no distress. Blood pressure was 140/84, pulse 88 and temperature normal. Funduscopic examination revealed some "silver wire" changes in the arterioles but was otherwise normal. Mouth showed partial edentia, gingivitis and periodontal disease. The tongue was deviated to the right. There was no goiter. The lungs were clear to percussion and auscultation, and the heart was normal. There were no organs or masses palpable in the abdomen. Examination of the extremities and neurologic examination were within normal limits.

Laboratory findings were hemoglobin 12.7 gm. per 100 ml., white count 4,100 cells per mm^3, differential count was 43 per cent polymorphonuclear cells, 54 per cent lymphocytes, 1 per cent monocytes and 2 per cent eosinophiles. Urinalysis revealed a specific gravity of 1.017, no albumin, 1+ sugar

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reaction, no acetone, and numerous white blood cells in the sediment. Levels of fasting blood glucose ranged from 202 mg. to 230 mg. per 100 ml. Blood urea nitrogen was 10 mg. per 100 ml. Liver function studies revealed a negative cephalin flocculation test, thymol turbidity 4 units, and bromsulfalein retention of 14 per cent in forty-five minutes. Tests of thyroid function were within normal limits. Serologic tests for syphilis were negative. Electrocardiogram and X rays of the heart and lungs were normal.

She was given a diet of 1,800 calories, with 225 gm. carbohydrate, 85 gm. protein, and 60 gm. of fat. Daily urinary glucose excretion and daily fasting and postprandial (two hours after lunch) blood glucose were measured. Blood glucose was determined by the method of Folin[11] and urinary glucose by the method of Benedict.[12] The patient's response to BZ-55 is shown in figure 1. During a six-day control period, the average twenty-four-hour glycosuria was 13.5 gm. (6.6 to 17.9 gm.), fasting blood glucose 211 mg. per 100 ml. (202 to 222 mg. per 100 ml.), and postprandial blood glucose 232 mg. per 100 ml. (200 to 272 mg. per 100 ml.).

Administration of BZ-55 was started on the seventh day with an initial dose of 2.5 gm. daily for seven days. The dose was increased to 4 gm. daily thereafter. On this dosage glucose excretion was reduced to 3 gm. (0.6 to 5.3 gm.), fasting blood glucose fell to 139 mg. per 100 ml. (106 to 187 mg. per 100 ml.) and postprandial blood sugar averaged 165 mg. per 100 ml. (132 to 197 mg. per 100 ml.). There was no change in weight, liver function tests or urinalysis during this time. Her leucocyte count on the eighth day of treatment was 4,300 cells per mm^3 with 31 per cent polymorphonuclear cells, 59 per cent lymphocytes, 4 per cent monocytes and 6 per cent eosinophiles. The blood level of BZ-55 was maintained at approximately 15 mg. per cent. On the seventeenth day of treatment she was discharged from the hospital on 4 gm. of BZ-55 daily and she returned to work.

Twice-daily qualitative urine sugar tests, done at home, remained negative. She remained asymptomatic until eight days following discharge, when she complained of fever, chills, anorexia and back pain. Except for a temperature of 102°, the physical examination was unchanged. The urine sediment was loaded with white blood cells. Because a urinary tract infection was suspected, a culture of the urine was obtained. She was given 1 gm. of streptomycin and 300,000 units of penicillin. The urine culture was reported subsequently to be sterile.

During the next three days her appetite returned, fever and back pain disappeared, and she felt much improved. On the following day, before returning to work, she was examined by one of us. She was asymptomatic, and physical examination revealed no new findings. Blood glucose four hours after breakfast was 126 mg. per 100 ml., and blood level of BZ-55 was 13.6 mg. per cent. Hematocrit was 37 per cent and a white count was 2,500 cells per mm^3 with 29 per cent polymorphonuclear cells, 47 per cent lymphocytes, 3 per cent monocytes and 21 per cent eosinophiles. In view of the leucopenia and eosinophilia, immediate but vain attempts were made to find the patient and discontinue the BZ-55.

The next day while waiting for a streetcar, she suddenly collapsed, and died within a few minutes. The gross pathologic findings at autopsy consisted of extensive hemorrhage into the scalp over the occiput, minimal subdural hemorrhage over the left hemisphere, pulmonary edema, myocarditis, and hypertrophic cirrhosis.*

Histologic examination was performed by Dr. Leon Sokoloff of this Institute. In the myocardium (right ventricle) was a focus of perivascular inflammation containing aggregates of eosinophilic polymorphonuclear leucocytes, metamyelocytes and

* We are indebted to Dr. Richard M. Rosenberg of the District of Columbia Coroner's Office, who performed the autopsy and very generously made tissue available for study.

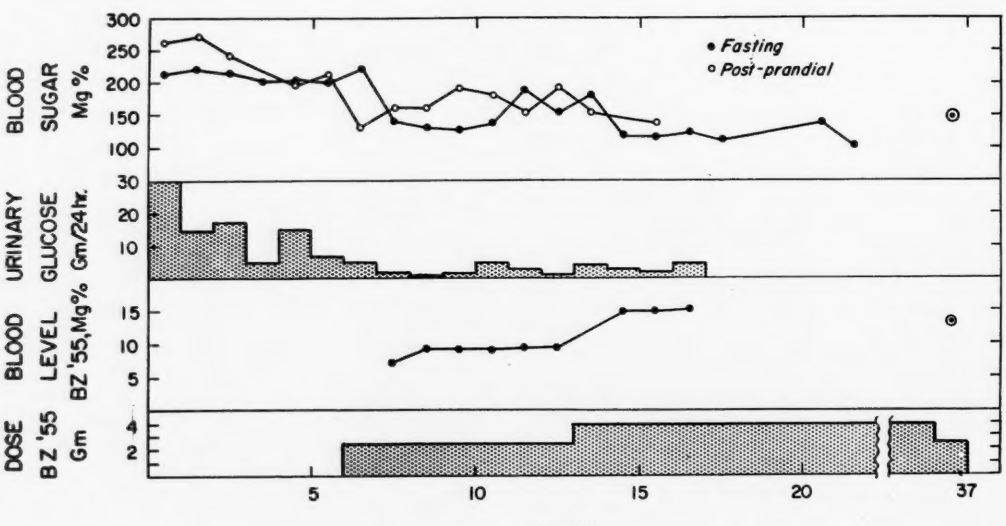

FIG. 1. Summary of patient's response to BZ-55.

mononuclear cells, including some Anitschkow myocytes and fibroblasts (figure 2). These cells lay among separated, collagenous fibers about a small artery. In the left ventricular myocardium there were numerous similar areas except for fewer eosinophiles. In addition there was a diffuse interstitial distribution of inflammatory cells. A proportion of the cells were undergoing fragmentation and there was some disintegration of collagen fibers.

The lungs contained focal, small infiltrates of mononuclear and plasma cells, and fewer eosinophiles about the walls of small bronchi. Many alveoli were filled with proteinaceous edema fluid. There was intensive congestion of alveolar capillaries about the moderate-sized pulmonary vessels.

The liver was the site of several pathologic changes (figure 3). In all portal areas there were infiltrates of chronic inflammatory cells, predominantly lymphocytes, and a scattering of eosinophiles, plasma cells, and possibly polymorphonuclear neutrophils. In some of these areas, there were miliary granulomata, consisting of large pleomorphic mononuclear cells and some pleomorphic multinucleated giant cells. No caseation was present. There was some proliferation of bile ducts and extension of vascular fibrous tissue from the portal area toward adjoining portal regions. Glycogen was preserved in about one-third of the hepatic cells (periodic acid Schiff stain). The portal lymph nodes showed numerous granulomatous lesions composed of plump mononuclear and epithelioid cells and pleomorphic multinucleated cells. Plasma cells were increased, but eosinophiles were rare. Special stains of liver and portal lymph nodes for bacteria (Brown-Brenn), acid fast organisms (Ziehl-Neelsen), fungi (periodic acid Schiff), and spirochetes (Steiner) were all negative.

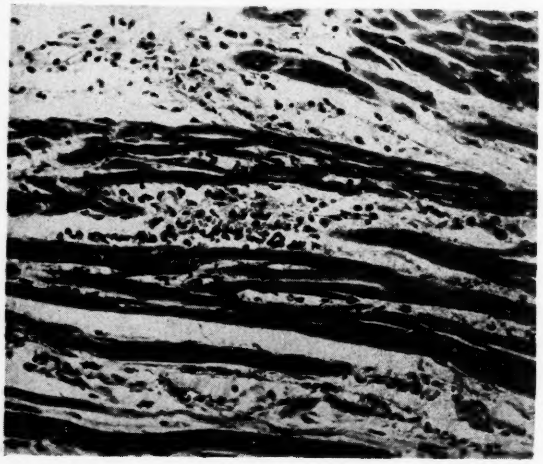

FIG. 2. Section from the right ventricle showing interstitial myocarditis.

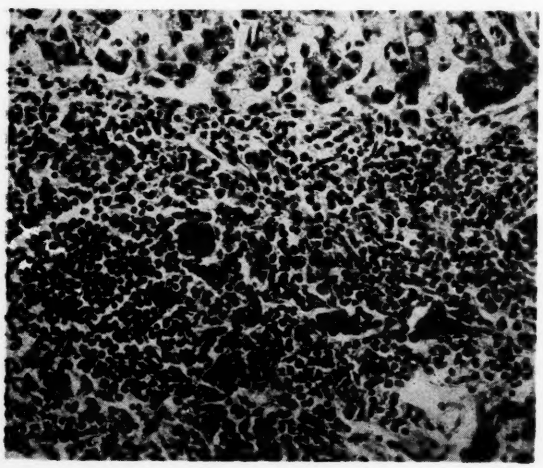

FIG. 3. Section from portal area of the liver revealing miliary granuloma.

The spleen contained similar miliary clusters of plump mononuclear cells at the margins of Malpighian bodies. There was only one small multinucleated cell. Small numbers of eosinophiles were present in the red pulp.

The pancreas was the site of marked postmortem autolysis. Some of the islets were partially to completely hyalinized. Chromalum hematoxylin phloxine stain failed to demonstrate alpha granules. This may have been due to postmortem change.

The kidney contained several small to moderate sized infiltrates of eosinophiles and mononuclear cells about some of the medullary tubules and veins just internal to the corticomedullary junction and in the wall of the pelvis. There were no crystals in the tubules.

In the esophagus, there were several miliary aggregates of mononuclear cells and lymphocytes about venules in the lamina propria. *The bone marrow* was hyperplastic, segmented neutrophils were infrequent and eosinophiles were more conspicuous.

The sections of the remaining tissues, including brain, contained no significant pathologic changes.

The final diagnoses were: 1) Hyalinization of islets of Langerhans with clinical diabetes treated with BZ-55; 2) mild portal cirrhosis of the liver; 3) sudden death probably due to hypersensitivity to BZ-55 with acute neutropenia and eosinophilia; interstitial myocarditis; granulomatous lesions in liver, spleen and portal lymph nodes; and focal infiltrations of eosinophiles and mononuclear cells in lung, kidneys, and esophagus; 4) pulmonary congestion and edema; 5) caseocalcific nodules, tracheobronchial lymph node; 6) mild to moderate generalized atherosclerosis.

DISCUSSION

Several features of this case suggest hypersensitivity to BZ-55. Unexplained eosinophilia, chills and fever have been reported in cases of sulfonamide toxicity,[8] and were present shortly before death in this patient. The lesion in the heart resembled that reported in cases of sulfonamide sensitivity that were common a decade ago, although eosinophilic leucocytes were not a conspicuous feature.[7] Granulomatous lesions were also present in such cases.[8, 9] Giant cells were not prominent in them as in the present instance. However, such giant cells

have been found in visceral granulomata in penicillin hypersensitivity in association with eosinophilic myocarditis.[10] Although this patient did receive one injection of penicillin four days before death, it seems likely that the changes were due to sulfonamide sensitivity. Eight days after beginning BZ-55 and seventeen days before the penicillin injection, a 6 per cent eosinophilia was present, contrasted with a maximum pretreatment value of 2 per cent. Chills, fever and back pain without apparent infection developed during treatment with BZ-55 and before penicillin was given.

The granulomatous changes in the liver, spleen and lymph node could conceivably have been produced by an infectious microbial agent, but multiple special stains did not demonstrate one.

Of the several possible causes of death in this case, hypoglycemia or arrhythmia seems most likely. However, hypoglycemic death is characteristically not sudden and Bertram[3] has not observed any instances of profound hypoglycemia in the course of clinical use of the sulfonylurea compounds. Of numerous blood sugars obtained in this patient, including one the day before death, none was in the hypoglycemic range. Since arrhythmias are not uncommon in association with myocarditis[13] this seems a more likely cause of death. The extensive myocardial infiltration by inflammatory cells and the severe pulmonary congestion and edema are consistent with this interpretation.

It thus appears that this sulfonamide derivative may be capable of producing some of the same toxic side effects as other sulfonamide compounds. Since these drugs are likely to be widely used, an awareness of this potentially fatal, toxic reaction is important.

SUMMARY

A case of sudden death in a diabetic patient receiving BZ-55 is reported. Chills, fever, neutropenia and eosinophilia were present shortly before death. Autopsy findings of interstitial myocarditis and focal miliary granulomata were similar to those previously reported in sulfonamide hypersensitivity.

ACKNOWLEDGMENT

We are indebted to Dr. Nicholas Leone of the National Institute of Dental Research for referring this patient for study and for his cooperation in her care.

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Effects of Carbutamide in the Diabetes Associated with Acromegaly

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In the course of our clinical investigation of carbutamide (BZ-55), we have had occasion to treat two patients who had both acromegaly and diabetes. In one of these patients, there was a prompt elimination of glycosuria and reduction in the fasting blood glucose without significant change in the two-hour postprandial

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blood glucose. The other patient showed no effect from 3 gm. of carbutamide daily and indeed, while on the drug developed acetonuria requiring resumption of insulin administration.

CASE REPORTS

Case One. A.L., a fifty-three-year-old woman, had had slowly progressive enlargement of the hands and feet for ten years and the diagnosis of acromegaly was made two and one-half years before admission to the Clinical Center. At that time she developed polyuria, polydipsia and burning of her eyes. Glycosuria and hyperglycemia were demonstrated, and the patient was given insulin. The dose was rapidly increased to 130 units per day, but glycosuria and acetonuria persisted, and the patient was hospitalized.

Large hands and feet, prognathism, enlargement of the sella turcica and hyperphosphatemia were found, and the patient was treated with thirty doses of x-radiation to the pituitary without appreciable amelioration of the diabetes. Since she was asymptomatic despite glycosuria and acetonuria, insulin was discontinued without incident.

At the time of her admission to the Clinical Center, she had noted no progression in the acromegalic symptoms. Despite constant glycosuria and acetonuria, she had no symptoms attributable to diabetes.

The main findings on physical examination were acromegalic features and healed choroiditis in the right eye. Urinalysis revealed a 4+ qualitative test for sugar and a positive test for acetone. Repeated determination of basal metabolic rate ranged from +1 to +19 per cent. Repeated serum phosphorus determinations varied from 2.9 mg. to 3.8 mg. per 100 ml. Other laboratory tests, including tests of liver function, were within normal limits. X-ray examination confirmed an enlarged sella turcica and acromegalic changes in the spine and digits.

The acetonuria disappeared within four days after she was given a 1,700 calorie diet. Her response to carbutamide therapy is shown in figure 1. Glycosuria, which averaged 17.4 gm. per day during the control period, rapidly disappeared while she was taking carbutamide. The control fasting blood glucose averaged 199 mg. per 100 ml. and fell to 149 mg. per 100 ml. during the period of carbutamide therapy. Postprandial blood glucose levels fell from an average of 248 mg. to 213 mg. per 100 ml. Because of comparatively low blood values of carbutamide the daily dose was increased to 2.5 gm. During the period of treatment there was no significant change in any of the laboratory examinations including urinary and blood corticoid levels. Before therapy, the protein bound iodine varied from 2.5 micrograms per cent to 5.1 micrograms per cent while during treatment it was 3.5 micrograms per cent. After eight days of carbutamide the drug was discontinued since it was not possible to follow the patient. Four days after omitting carbutamide, she was discharged from the hospital. At this time she was still aglycosuric but her fasting blood sugar values had begun to rise toward the pretreatment level.

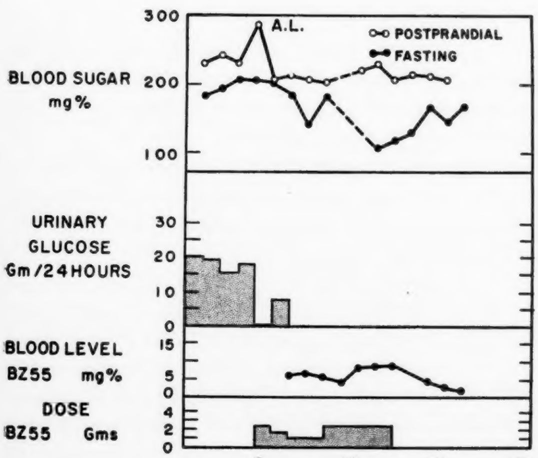

FIGURE 1

Case Two. B.G., a thirty-eight-year-old female, developed symptoms of diabetes eight years prior to admission to the Clinical Center. At that time the diagnosis of acromegaly was also made on the basis of her physical features and an enlarged sella turcica by x-ray examination. She was given a diet and 30 units of insulin daily, but after three months, the insulin was discontinued. However, after six months without insulin therapy, her glycosuria recurred and insulin was again administered. Over the next seven years her daily insulin dosage was increased to 120 units. Since her acromegaly was asymptomatic, she had not received any therapy for it. At the time of her admission to the Clinical Center she was asymptomatic except for occasional insulin reactions and moderate headache.

The positive findings on physical examination were blood pressure 170/100 mm. Hg, prognathism, enlargement of the hands and feet, and macroglossia. The thyroid was enlarged (estimated twice normal size) and the liver edge was felt one centimeter below the costal margin. Urinalysis revealed 2+ test for sugar, but was otherwise normal. The serum phosphorous ranged between 4.9 mg. and 3.9 mg. per 100 ml. Other laboratory examinations including tests of thyroid and adrenal function were within normal limits. X-ray examination disclosed an enlarged sella turcica and acromegalic changes of the hands and feet.

The patient was given an 1,800 calorie diet (carbohydrate, 200 gm.; protein, 60 gm.; fat, 85 gm.) and insulin was withdrawn for eight days preparatory to the administration of carbutamide. Figure 2 summarizes the response to carbutamide. Glycosuria averaged 110 gm. per day during the control period. Insulin was given for three days before the institution of carbutamide when the diabetic control began to deteriorate, but was then withheld for two days while carbutamide was given in doses of 3 gm. per day. During this time her glycosuria increased and acetonuria developed. There was a concomitant rise in her fasting blood sugar. Because of the deterioration of her diabetic control, insulin therapy was begun without changing her dose of carbutamide. Over several days her insulin dosage was stabilized at approximately 95 units of insulin with a marked reduction in her glycosuria and blood sugar values. Carbutamide was discontinued after sixteen days, without any apparent increase in her insulin

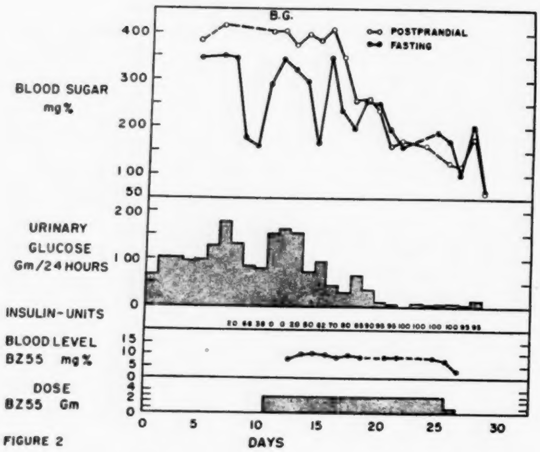

FIGURE 2

requirement. During treatment with carbutamide there was no significant change in any of the laboratory tests, renal, thyroid, adrenal and liver functions.

DISCUSSION

It is impossible to draw any conclusions from these contrasting cases regarding the efficacy of carbutamide in the diabetes which accompanies acromegaly, particularly since both such diabetes and the action(s) of the sulfonylurea drug are incompletely understood. The second patient, B.G., who did not respond, had developed diabetes at age of thirty, and developed ketonuria when insulin was withheld. Further, she had been treated with insulin for over eight years. These several factors have been shown to influence adversely the chances of success with the sulfonylurea agents. In the first patient, despite poor regulation with as much as 130 units of insulin previously, carbutamide was highly effective. She was able to do without insulin without developing acetonuria, but had been treated with insulin for a short time only. In addition to these differences, it is possible that the diabetes of acromegaly is influenced by extrapituitary factors. In this connection it is to be noted that there was no observed change in thyroid or adrenal function in these patients during carbutamide therapy.

SUMMARY

Two patients with diabetes and acromegaly were studied using carbutamide. One patient made a satisfactory response to the drug while the other one did not.

Occurrence of Sensitivity and Side Reactions Following Carbutamide

W. R. Kirtley, M.D., Indianapolis*

Toxicity has been an item of primary concern from the beginning of the clinical trial program with carbutamide. Knowing that as a class sulfonamide drugs cause side reactions of certain types, we were alerted to the possibility of complications developing and a request for information in this regard was included in the report sheets furnished to all investigators.

Upon receipt of information from the National Institutes of Health concerning the first fatal case, just reported by Field and Federman, it became apparent that this information must be supplied to all physicians who had been receiving the drug on clinical trial. This decision was reinforced by two additional fatal cases reported from New York City. Although the data were not clear-cut, there was at least some indication that the administration of carbutamide might have contributed to the deaths in these cases as well.

At the time the information was dispatched, a request for data as to the incidence of toxicity of any sort was included and a tabulation of the data received is shown in the following tables.

At the present time, carbutamide has been supplied to 2,900 physicians. It is assumed that the majority of this group now has experience with the drug in the treatment of diabetes. This report is derived from data which have been obtained from almost half of this group and the total sample includes over 7,000 patients. (The unfavorable reaction percentage has been determined to be a bit over 5 per cent.)

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TABLE 1

Reaction tabulations

Questionnaires returned	1,319
Cases reported	7,193
Side reactions	389
Reaction per cent	5.36

TABLE 2

Reactions reported following use of carbutamide

	No. cases		No. cases
Rash	109	Acute edema	4
Anorexia, nausea, vomiting	81	Crystalluria	3
Malaise, lethargy, fever	79	Thrombocytopenia	2
Agranulocytosis, leukopenia	58	Purpura	2
General allergy	25	Eosinophilia	2
Cardiovascular, renal	16	"Headache"	2
Exfoliative dermatitis	6	Psychosis	1
Acute anemia	6	Hypothyroidism	1
Jaundice, liver function	6	Methemoglobinemia	1
		Tachycardia	1
		Sudden myocardial failure	1
		Unclassified	10

TABLE 3

Incidence of sensitivity to other sulfonamides

1) Topical Application (Sulzberger, et al.)	
Na-Sulfadiazine	57%
Sulfanilamide	22%
Sulfathiazole	7%
Sulfadiazine	5%
2) Systemic Reactions	
Sulfathiazole (Kent and Diefendorf)	10%
Sulfadiazine (2nd Course) (Lyons and Balberor)	16%
"Sulfonamides" (Hasking and Lawrence)	5%

TABLE 4

Fatalities following carbutamide

Sudden myocardial failure	1
Sulfonamide sensitivity	2*
Bone marrow depression	1
Dermatitis exfoliativa	1*
Acute pulmonary edema	1
Liver involvement	2
	8

* Autopsy data not yet available

No attempt has been made to screen the reports as to the validity of the claim for side reaction. In many instances the report indicated nonspecific, subjective complaints which, for lack of a better classification, are here listed as "general allergy." It is to be noted, however, that the majority of side reactions can be considered as those associated with sulfonamide drugs. A certain percentage of reaction of these types might have been expected.

In the tabulation, hypoglycemic reactions were not included since this effect cannot be considered as a toxic side reaction.

The total incidence of toxic effects, if examined uncritically, is a cause for some concern, particularly if the drug is to be given over a long period of time. It is important to know the relative incidence of side reaction, as compared to other sulfonamide drugs.

Although reactions following topical application cannot be compared directly with systemic reactions, these data are included for completeness.

At the time of this report, eight fatalities have occurred under circumstances which might indicate that the drug had contributed.

The data are incomplete in most cases but it is sufficiently significant to set these cases apart from ten additional deaths reported in patients receiving carbutamide in which the drug cannot be implicated.

At the present time, no definite conclusions can be drawn and the information is presented as a progress report. Undoubtedly, sensitivity reactions do occur, and it is anticipated that on final analysis the incidence will not exceed 5 per cent.

COMMENT

PAUL N. HARRIS, M.D., (*Indianapolis*): I have examined sections of tissues from five individuals who have died following administration of BZ-55. The first case was the one described by Dr. Field. The second showed similar, but less intense reaction, i.e., myocarditis and granulomatous lesions in some of the abdominal viscera. Tissues from the third patient showed no granulomatous lesions, but there were a few foci of infiltration of small numbers of lymphocytes in the myocardium. I am not sure that the myocardial lesion is properly attributable to BZ-55. The fourth patient developed agranulocytosis with no lesions in the myocardium or abdominal viscera. The findings in the fifth patient will be described by Dr. Howard Root; I shall not anticipate his discussion, but consider it sufficient to say that the lesions present are entirely different from those in the other four patients. The development of myocarditis, granulomatous lesions, and agranulocytosis has been reported following administration of other sulfonamides, and also of various unrelated substances.

Clinical Experience with Carbutamide (BZ-55)

A Progress Report

Rafael Camerini-Davalos, M.D., Howard F. Root, M.D.,† and Alexander Marble, M.D.,‡ Boston*

From December 1955, through August 1956, we used sulfonylurea compounds in 620 patients with diabetes. Of this number 380 received BZ-55, 230 were given Orinase and 10 SPTD (sulfapropylthiodiazole). In the present paper our experience with BZ-55 alone is reported. This is in the nature of a progress report; a more complete and detailed presentation of results will be made at a later date.

Among the 380 who have received BZ-55, 187 patients, including 101 of fifteen years of age and under, were studied only following a single administration of the drug in the course of a response test (see below). The remainder, 193 patients, was observed while being maintained on BZ-55. Of these, in sixteen cases the period of observation was insufficient, in twenty-four the drug was discontinued because of complications and in three the preparation was stopped for other reasons. The results in the remaining 150 patients form the basis of the present discussion. These patients have been maintained on BZ-55 for periods varying from less than one month (thirty-one cases) to more than six months (twenty cases).

Of the 150 patients, 66 were males and 84 females. Most of them (116, or 77 per cent) were between the ages of 40 and 70 years; 14 were under 40 and 20 over 70. Diabetes had been present in 42 (28 per cent) of the group for 10 to 20 years; in 99 (66 per cent) the duration was under 10 years and in 9 (6 per cent) over 20 years. Of the 150 patients, 65 had never received insulin and 75 had been taking less than 40 units daily.

RESPONSE TEST

It has become our practice not to administer the drug without first carrying out a four-hour response test following the procedure listed below:

1. In patients requiring insulin, either omit insulin or give only crystalline insulin in the forty-eight hours preceding the test.
2. Omit insulin and breakfast on the day of the test.
3. Determine the blood sugar in the fasting state.
4. Give 3.0 gm. of BZ-55 orally.
5. Determine the blood sugar four hours after giving the drug.

In many patients the blood sugar was determined also at two, six and eight hours after the administration of BZ-55, but experience has shown that the four-hour value is the most helpful. In interpreting the results we have regarded a fall in blood sugar of more than 20 per cent as a good response. A decrease of 20 per cent or less has been rated as unsatisfactory. In general the results of the response tests have served a good, although by no means infallible, guide as to whether satisfactory control of blood sugar might be anticipated with maintenance of the patient on the preparation. As may be seen in table 1, of forty-eight patients with a good response test, forty-one achieved good, and five fair, control of blood sugar and glycosuria in long-term maintenance studies. On the other hand, of thirteen patients with a poor response test, nine had poor control in maintenance trials. It must be admitted that occasionally patients are encountered who achieve good control in maintenance studies despite a poor outcome during a response test. Furthermore, some patients who show little or no benefit from the drug at first may, after a period of time, experience good control of blood sugar and glycosuria, particularly if some complication such as an infection, has been overcome.

MAINTENANCE STUDIES

Methods. The maintenance studies were initiated both with patients in the hospital and with those seen as outpatients. Insulin was almost invariably stopped although in certain cases reduction in dosage was carried out gradually. In only seven patients was insulin given regularly in reduced dosage along with BZ-55. The usual sequence of study was as follows: (a) if the patient had

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This study was aided by a grant from Eli Lilly and Company.

Carbutamide (BZ-55 Aminophenurobutan Lilly) was furnished by Eli Lilly and Company.

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been treated with a depot insulin, this variety was discontinued and either no insulin or only crystalline insulin was given for two to five days; (b) a response test was performed; (c) if this was positive, then an attempt was made to control hyperglycemia and glycosuria with BZ-55 alone. The dosage of BZ-55 usually was 3 gm. daily for two or three days and finally 0.5 to 1.5 gm. daily thereafter. Attempt was made to keep the amount down to 0.5 to 1.0 gm. daily. Usually the entire dosage was given before breakfast. The diet was kept at a constant level throughout the period of study; in the adult patients, this consisted usually of 150 to 190 gm. of carbohydrate, 70 to 90 gm. of protein and 60 to 80 gm. of fat, yielding from 1500 to 1900 calories daily.

In hospitalized patients the amount of sugar excreted in the urine in twenty-four hours was determined daily and qualitative tests made before each meal. Frequent determinations of the blood sugar were made before each of the three meals.

With outpatients living at home, attempts were made to have the urine tested qualitatively before each meal although complete cooperation in this regard often was not secured. Patients were seen at intervals of one to two weeks at first, and later at monthly intervals so that determinations of the blood sugar could be made and the physical status of the patient evaluated.

The degree of control obtained during maintenance studies was classified as "good," "fair," or "poor" according to the standards outlined in table 2. In practice, the most commonly used criteria were as follows: If in a given case, 70 per cent of blood sugar ("true glucose") values were 110 mg. per 100 ml. or below either in the fasting state or three hours after a meal, and if the twenty-four-hour specimen of urine contained 2 gm. or less of sugar, then "good" control was judged to have been secured. If the corresponding values were 130 mg. per 100 ml. and 5 gm. for blood sugar and urine sugar, respectively, then the degree of control was classed as "fair."

Results. Using the standards outlined in table 2, the results of maintenance studies in the 150 patients were classified as shown in table 3. It must be pointed out that, with seven exceptions, patients were maintained on BZ-55 alone, without the use of insulin.

It is evident that in 86, or 57 per cent, good control and in 18 patients, or 12 per cent, fair control, was obtained. In 41 patients, 31 per cent of the total, poor results followed the use of BZ-55.

After satisfactory control of hyperglycemia and glycosuria had been secured for two months or more, in twenty-six cases BZ-55 was discontinued and studies carried on as before in order to determine whether or not the beneficial effect had been due simply to long-continued dietary control alone. It was found that in 16 of the 26 cases, it was necessary to resume BZ-55 because of rising blood sugar values and return of glycosuria.

In ninety-three patients white blood counts were done at various times during the administration of BZ-55; no instance of leucopenia was encountered. Eosinophilia occurred in one patient. In seventeen patients red blood counts, in forty-six cases blood hemoglobin determinations and in thirty-five cases blood platelet counts were carried out; the results were uniformly normal except in one patient who developed a well-marked anemia.

In eighty-seven patients the blood level of BZ-55 (expressed as sulfanilamide) was determined. In patients

TABLE 1

Comparison of response test with results of treatment up to nine months

BZ-55 response test		Clinical control		
Results	Patients (no.)	Good	Fair	Poor
Good	48	41	5	2
Poor	13	1	3	9
	61	42	8	11

TABLE 2

Standards of control*

	Degree of control†				
	Good		Fair		Poor
Relation to food	Blood sugar‡ mg./100 ml.	Urine sugar per cent	Blood sugar‡ mg./100 ml.	Urine sugar per cent	All other cases
Fasting	110	Trace	130	0.1	
1 hr. P.C.	150	0.3	180	0.5	
2 hr. P.C.	130	0.1	150	0.3	
3 hr. P.C.	110	Trace	130	0.1	
Urine sugar in 24 hr.	2 gm. or less		5 gm. or less		

* For purpose of classification as to degree of control, 70 per cent or more of values must conform with standards listed in the table.

† These standard values are the highest acceptable.

‡ Glucose as determined by the Somogyi-Nelson procedure.

TABLE 3

Degree of control of diabetes obtained with BZ-55

	Patients	
Control	Number	Per cent
Good	86	57
Fair	18	12
Poor	46	31
	150	100

responsive to the drug as regards blood sugar, blood sulfanilamide levels in the range of 8 to 12 mg. per 100 ml. were found effective. However, similar blood levels were obtained in individuals not responsive to BZ-55.

SEQUELAE

In 26 or 9.2 per cent of 279 patients receiving BZ-55 (380 patients less 101 children who had a response test only), untoward effects or sequelae were observed. These are listed in table 4.

TABLE 4

Sequelae in 279 patients on BZ-55 (excludes 101 children with response test only)

Skin rash	15	Diarrhea	2
Jaundice	4	Disorientation	1
Hypoglycemia	2	Cer. vasc. accid.	2
Anemia	1	Par. auric. tach.	1
Nausea with or without vomiting	5	Total	33

33 sequelae in 26 patients or 9.2 per cent

A skin eruption occurred in fifteen patients. The most common form was a measles-like rash affecting particularly the body but occasionally the face. In some instances only a generalized erythema was seen, but often the eruption was elevated. The rash was in some cases accompanied by fever as high as 103° F. In only a few patients did itching occur. In two patients, enlargement of lymph nodes preceded the onset of fever and skin eruption. With these symptoms general malaise and nausea often were present. Unfortunately, in almost every patient who developed a skin eruption, it was found that the patient was also receiving other medication, including multivitamin preparations and, in some cases, barbiturates and antibiotics. Antihistaminics were, in general, ineffective in treatment of the rash.

Jaundice occurred in four patients. In two it was mild and of short duration. In one of these patients, carcinoma of the prostate was under treatment. In these two cases, possibly the jaundice had no relation to the drug. In the third case of jaundice, BZ-55 had been taken for two weeks. When the patient returned for observation he had fever and a generalized skin eruption together with jaundice. He had been given by other physicians Terramycin®, methamphetamine, Chlor-Trimeton®, ascorbic acid, salicilamide and Coricidin Forte®. The jaundice persisted and only after two months was the urine found free of bile. In this case a biopsy of the liver revealed areas of necrosis of the central type with marked bile stasis. In the fourth case, a woman aged fifty-eight years with mild diabetes of some ten years' duration, BZ-55 was begun at her own request. Loss of appetite, weakness and jaundice appeared in the fourth week and she noted swollen cervical nodes at the onset of the illness. In her case as in the third case of jaundice, a high serum alkaline phosphatase level, with little alteration in the cephalin flocculation and other liver function tests, was observed. She had no fever and her progress seemed satisfactory until suddenly she became comatose; a severe hemolytic anemia developed, although no anemia had been present on admission to the hospital several days previously. Death occurred suddenly. The post-mortem examination, which was complete and included the brain, showed two important findings. The liver presented general hepatitis without any cholangitis. The hepatitis was characterized by multiple areas of necrosis with marked bile stasis. The kidneys showed a typical bilirubin nephrosis of such degree that in the opinion of the pathologist, kidney failure might well have been the chief cause of death. In addition there was a mild degree of chronic vascular nephritis.

Severe *hypoglycemia* occurred twice. In one man, despite persistence for forty-eight hours, recovery took place. Another man who had received BZ-55 in dosage of 3 gm. for the first six days and 2 gm. on the last day, developed hypoglycemia with a blood sugar as low as 38 mg. per 100 ml. Following omission of the drug for two days, the blood sugar rose to 246 mg. per 100 ml. BZ-55 was then resumed and good control was obtained with 2.0 gm. daily. On the twelfth day severe diarrhea began. On the fourteenth day a blood sugar of 46 mg. per 100 ml. was observed. The drug was then omitted again although only 1.0 gm. per day was being given at this time. The next day the blood sugar reached levels of 164 and 171 mg. per 100 ml. but two days later the patient died, probably because of myocardial failure. Permission for autopsy was not obtained.

In one patient a severe *anemia* was observed together with malaise and grippe-like symptoms. Recovery was uneventful. *Nausea* with or without *vomiting* occurred in five cases and *diarrhea* in two. In one elderly woman, eighty years of age, a period of *disorientation* was observed when the blood sulfanilamide level reached 21 mg. per cent.

Certain complications occurring in patients receiving BZ-55 were most likely not related to the administration of the drug. Thus, in two cases *cerebrovascular accidents* occurred; recovery was rapid in one, but the second patient was an elderly woman with hypertension of

long duration and death followed what seemed a typical cerebral hemorrhage. In one patient *paroxysmal auricular tachycardia* occurred with good recovery.

SUMMARY

1. From December 1955 through August 1956, BZ-55 was administered to a total of 380 patients. Of this number, 150 patients were maintained on the drug for periods ranging from a few days to nine months.

2. The results of the response of the blood sugar to a single dose of BZ-55 have served as a good guide as to whether satisfactory control of hyperglycemia and glycosuria might be anticipated in maintenance studies.

3. According to arbitrarily chosen standards of control, 57 per cent of the 150 patients achieved "good," and 12 per cent "fair" control of hyperglycemia and glycosuria. In 31 per cent of cases, "poor" control was obtained and the drug discontinued.

4. In 26 or 9.2 per cent of 279 patients, untoward effects or sequelae were noted. These included skin eruptions in fifteen patients, jaundice in four, severe hypoglycemia in two, anemia in one, nausea with or without vomiting in five, diarrhea in two and disorientation in one patient. In addition, two patients developed cerebrovascular accidents and one paroxysmal auricular tachycardia while receiving BZ-55. In two of the four cases with jaundice, severe liver damage occurred with one fatality.

Clinical Experience and Experimental Studies with Tolbutamide

James W. Craig, M.D., and Max Miller, M.D., Cleveland

This conference affords evidence of the intense interest and the thoughtful investigations which have been stimulated by the recent introduction of two sulfonylurea compounds, carbutamide and tolbutamide. A careful study of the effects of these compounds on patients with diabetes mellitus of varying types and etiologies should aid in the assessment of their clinical usefulness and might yield information about their blood sugar lowering mechanism. Such a study will be described in this paper. Detailed descriptions of some of our results have been published previously[1, 2] and will be only summarized here.

CLINICAL STUDIES

All of the patients were hospitalized and maintained on a constant dietary intake during the period of study. When insulin was employed it was given as regular insulin twice daily, except in the case of the patient with lipoatrophic diabetes who received 500 units of insulin each day in a single dose. Tolbutamide* was the sulfonylurea compound used for these studies.

The first three patients had diabetes mellitus of undetermined etiology but varied in their clinical characteristics. Each one represented a different type of R. D. Lawrence's three types of idiopathic diabetes mellitus.[3] As previously reported, in a patient with stable or lipoplethoric diabetes, who had required 45 units of NPH insulin daily, the fasting blood glucose concentration and twenty-four-hour glucose excretion were well controlled without insulin by 2 gm. of tolbutamide daily. It is interesting that surgery on this patient was associated with a temporary marked rise in the blood concentration and urinary excretion of glucose in spite of the continued administration of tolbutamide. This patient has now received the drug, 2 gm. daily for ten months, with no apparent decrease in its effectiveness and without toxic manifestations. The results in a patient with labile or insulin deficient diabetes were in sharp contrast to those just described. During the period of study this patient received 70 units of insulin daily instead of her usual dose of 90 units to ensure a persistent hyperglycemia and glycosuria. The administration of 2 gm. of tolbutamide daily in addition to 70 units of insulin produced no significant alteration in the blood glucose concentration or the glucose excretion. The third patient had lipoatrophic diabetes mellitus and required 2,000 units of insulin daily to control her hyperglycemia. Two grams of tolbutamide daily produced a significant lowering of the fasting blood glucose concentration and twenty-four-hour glucose excretion in the absence of exogenous insulin, but adequate clinical control was not obtained with this drug alone. The effect

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*Orinase® was supplied by The Upjohn Company.

of this dose of tolbutamide on the fasting blood glucose concentration was equivalent to that of 500 units of insulin, but the effect on the glycosuria was much less marked.

The next two patients furnish examples of diabetes mellitus which was secondary to surgical removal of pancreatic tissue. The first of these patients had developed mild, stable diabetes following subtotal pancreatectomy; it was estimated that 80 per cent of the pancreas had been removed. He required 4 to 14 units of regular insulin daily. In the absence of exogenous insulin, 2 or 3 gm. of tolbutamide daily produced a definite decrease in the fasting blood glucose concentration; although the glycosuria was decreased, it was not as well controlled as was the fasting blood glucose concentration. This observation suggested that the drug has its major effect on processes which maintain the concentration of blood glucose in the fasting state, while an influence on the disposal of exogenous glucose is absent or less evident. This effect was also illustrated by a study of his blood glucose changes following the intravenous administration of glucose and fructose. Tolbutamide diminished the blood glucose rise which was associated with fructose administration, but did not alter the rise which was produced by giving glucose. The second of these patients was a thirty-six-year-old woman who developed diabetes mellitus following total pancreatectomy. There was no family history of diabetes mellitus and the patient was quite thin. For twenty years she had had symptoms of chronic pancreatitis. On April 18, 1956, a total pancreatectomy was performed; the excised organ was the site of severe chronic fibrous and calcific pancreatitis. Following pancreatectomy, the patient developed diabetes mellitus which was characterized by marked lability with the occurrence of both ketosis and hypoglycemia, and required up to 60 units of insulin daily. A trial on tolbutamide was conducted in June and July, 1956. During the control period, the daily insulin dose was reduced to 25 units to ensure a persistent hyperglycemia and glycosuria; crystalline insulin was given before breakfast and supper. As shown in table 1, the administration of 2 gm. of tolbutamide daily for eleven days in addition to 25 units of insulin did not produce a significant alteration in the fasting blood glucose concentration or the glucose excretion. It is interesting that no potentiation of the effect of exogenous insulin was demonstrated. Such a finding casts some doubt upon the possibility that tolbutamide exerts its effect by an inhibitory action upon an insulin antagonist or destroyer.

TABLE 1

Effect of tolbutamide in a totally pancreatectomized patient

Material	Number of days	Glucose: Fasting blood mg./100 ml.	Glucose: Urine gm./day
Insulin	11	433±61 (S.D.)	22±12
Insulin+ Tolbutamide	11	435±36*	31±13†

* $p>0.5$
† $p=0.1$-0.05

Another instance in which diabetes mellitus may have been due to pancreatic damage, presumably with insulin deficiency, was that of a sixty-seven-year-old man with generalized hemochromatosis. Diabetes mellitus had been diagnosed in 1944 and the diagnosis of hemochromatosis had been confirmed by a liver biopsy in 1949. He had recently been maintained on 55 units of NPH insulin daily. The effect of tolbutamide administration in this case was studied at the Veterans Administration Hospital in Cleveland, Ohio, by Drs. Reginald A. Shipley and Paul E. Wisenbaugh. When the insulin dose was reduced to 25 units daily, marked hyperglycemia and glycosuria occurred. The addition of tolbutamide in doses up to 4.5 gm. per day had no demonstrable effect on blood or urine glucose content. A single intravenous dose of 2 gm. of sodium tolbutamide produced no lowering of the blood glucose concentration 4.5 hours after injection.

The next two patients had diabetes mellitus from increased amounts of adrenal cortical steroids being present. The first of these patients was a fifty-four-year-old woman with classical Cushing's syndrome, diagnosed first in 1953. In 1954 classical symptoms and signs of diabetes mellitus appeared and insulin therapy was started. She had recently been taking 24 units of insulin daily. As shown in figure 1, tolbutamide reduced the fasting blood glucose concentration and urinary glucose excretion both before and after the removal of an adrenal cortical adenoma. A fifty-two-year-old man with bilateral idiopathic optic neuritis developed hyperglycemia and glycosuria while receiving 300 mg. of prednisolone daily as therapy for the ophthalmologic condition. His family had no history of diabetes mellitus, and he was not obese, but he required as much as 40 units of insulin daily. At a time when the dose of prednisolone was reduced to 150 mg. and he also received 40 units of ACTH gel daily, he was given a single intravenous dose of 2 gm. of sodium tolbutamide. Within three hours his blood glucose fell from 231 mg. per 100 ml. to 137 mg. per 100 ml. The effect of tolbutamide administration over a longer period of time was not studied in this case of steroid diabetes. Thirty days after the last dose of prednisolone

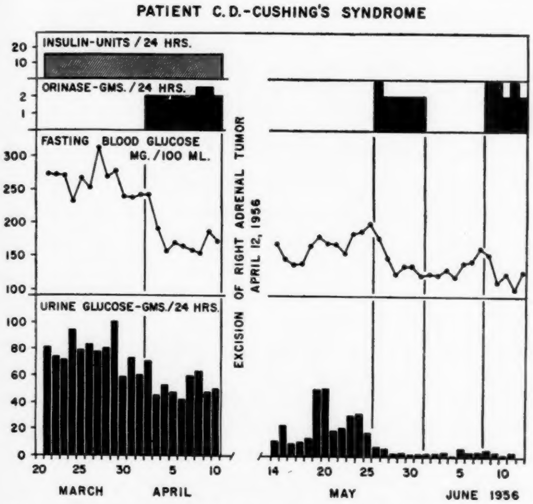

FIG. 1. Effect of tolbutamide on fasting blood glucose concentration and 24-hour glucose excretion in a patient with Cushing's syndrome before and after removal of an adrenal cortical adenoma. During the postoperative period the patient received 25 mg. of cortisone daily.

and seventeen days after ACTH had been discontinued, the intravenous administration of 2 gm. of tolbutamide produced a fall in the blood glucose concentration from a fasting level of 64 mg. per 100 ml. to 42 mg. per 100 ml. in thirty minutes; at the end of three hours the blood glucose level was 74 mg. per 100 ml. It appears that the presence of increased amounts of adrenal cortical steroids does not interfere significantly with the hypoglycemic effect of tolbutamide.

SUMMARY AND CONCLUSIONS

1. The blood sugar lowering effect of tolbutamide has been studied in patients with diabetes mellitus of varying types and etiologies. A blood sugar lowering effect of the drug was demonstrated in a partially pancreatectomized patient, but hyperglycemia and glycosuria were not reduced by tolbutamide in a totally pancreatectomized patient who received a constant dose of insulin during the period of study. The drug was effective in decreasing the blood glucose concentration in a patient with Cushing's syndrome, including diabetes mellitus, and in another patient with steroid diabetes secondary to prednisolone administration.

2. Some suggestions regarding the mechanism of action of tolbutamide have been made on the basis of clinical observations. The drug appears to diminish the endogenous formation of glucose without altering the rate of utilization of administered glucose. An inhibitory effect upon an antagonist or destroyer of insulin seems unlikely. The action of the drug is not altered significantly by adrenal cortical steroids.

ACKNOWLEDGMENT

The authors wish to thank Drs. Charles Christian and Lawrence Hutchison for their valuable assistance in the study of some of the patients described in this paper.

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The Influence of Carbutamide on Thyroid Function in Older Men and Women with Diabetes Mellitus

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Long-term therapy is implied in the application of the hypoglycemic action of carbutamide to diabetes mellitus. The presence of a sulfonamide grouping in this sulfonylurea raises the question whether or not its continued use may lower thyroid function. The present studies were undertaken in an effort to answer this question.

PROCEDURE

Twenty-seven patients with diabetes mellitus, thirteen men and fourteen women, with ages ranging from 57 to 85, average 70.8 years, were chosen from the wards of Bird S. Coler Hospital. Most of these were taking insulin at the time the study was begun. However, none of the subjects selected could be considered fully controlled, although none was completely out of control. Periods of treatment ranged from three to twenty-one weeks, with additional observations up to fourteen weeks after therapy was discontinued.

All subjects had been on the wards of the hospital for more than six months before the beginning of the study, and had been under the care of the same resident and nursing staff for three or more months when observations were started.

The known duration of the diabetes mellitus varied from six months to thirty-five years with an average duration of 12.1 years and the use of insulin from zero to thirty-five years with an average of 8.6 years. The earliest age at onset was thirty-nine years, so it is clear that all of these subjects might be looked upon as being of the adult or stable type. One subject showed features of the brittle type. He was not well controlled with 70 units of insulin and had had frequent episodes of hypoglycemia and acidosis prior to the initiation of therapy.

Thyroidal function was appraised by determinations of the I^{131} uptake by the thyroid gland, basal metabolic rate and serum protein-bound iodine. In this older age group the clinical condition of the patient was of lesser value. Tracer doses of I^{131} never exceeded twenty-five microcuries each, nor was any patient given more than a total of one hundred microcuries in experimental periods up to forty weeks in length. Except for the four-day studies, a radioiodine tracer dose was not repeated under three weeks. The final percentage uptakes were calculated from the throat and urine counts.

RESULTS AND COMMENTS

A. *Radioiodine Studies*

Three separate approaches were made to the influence of carbutamide on the uptake of radioiodine by the thyroid: (1) uptake gradients were determined in two subjects according to the method of Stanley and Astwood;[1] (2) large doses of the drug (4.0 gm. and 3.0 gm.) were given daily for four days to six and three patients, respectively, and the initial uptakes compared with those at the end of the period; and (3) uptake of tracer doses of radioiodine was determined in sixteen subjects before, at intervals during, and one or more times following the long-continued use of therapeutically effective doses of carbutamide.

1. *Uptake gradients of I^{131} following the administration of carbutamide.* Two subjects were given a single 4 gm. dose of carbutamide as soon as the individual

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uptake gradient for a preceding dose of I^{131} had been established. No change occurred in one subject and suppression of uptake was complete for twenty-four hours in the other. This experiment left us with the impression that carbutamide is capable of depressing the uptake of iodine by the thyroid in some individuals under certain conditions. To study the question further as to whether or not suppression of thyroid function can be readily produced by carbutamide we tried the effects of daily doses of carbutamide for four days on the uptake of iodine by the thyroid.

2. *The influence of four large daily doses of carbutamide upon the thyroid uptake of iodine.* Ten subjects were used for this study. Seven of them were given 4 gm. daily for four days and three, 3 gm. daily for a like period of time. The results are shown in figure 1. Of the seven subjects given 4 gm. daily, one had only a 3 per cent uptake prior to using the drug; therefore, data obtained from him have been eliminated from the final calculations. In one subject the initial uptake was 12 per cent but in the remaining five, values were normal, varying from 19 to 31 per cent. For the six subjects, the average pretreatment uptake of I^{131} was 23.3 per cent. After four days of treatment with 4 gm. daily, the thyroid uptake in these six patients ranged from 2 to 10 per cent with an average of 5.3 per cent (figure 1).

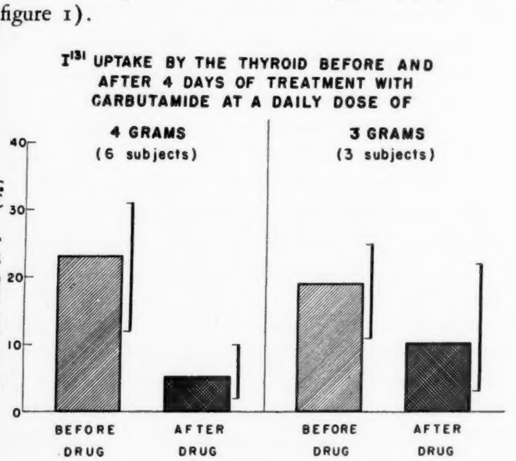

FIGURE 1

3. *The influence of the long-continued use of 1.0 and 2.0 gm. daily of carbutamide upon the thyroidal uptake of radioiodine.* Sixteen subjects participated in this part of the study. After the initially larger starting doses, ten were continued on 1.0 gm. of carbutamide daily and six on 2.0 gm. daily. For those using 1.0 gm. daily, a dose of 3 gm. was given on the first day, 2 gm. on the second, and 1.0 gm. daily thereafter. For the patients continued on 2 gm. daily, 4 gm. were given each of the first two days, 3 gm. on the third and fourth days, respectively, and 2 gm. daily thereafter.

Half of the subjects were retested at the end of two weeks, and half at the end of three weeks of therapy. All of the subjects were tested again after nine weeks of treatment except for one patient who died as a result of a thrombosis of his inferior vena cava at the end of the third week.

The results of these I^{131} uptakes are graphically depicted in figures 2 and 3.

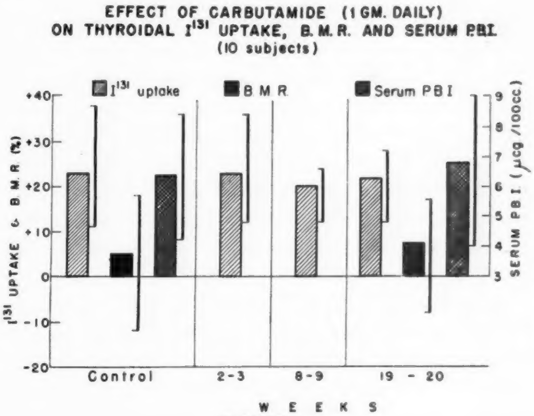

FIGURE 2

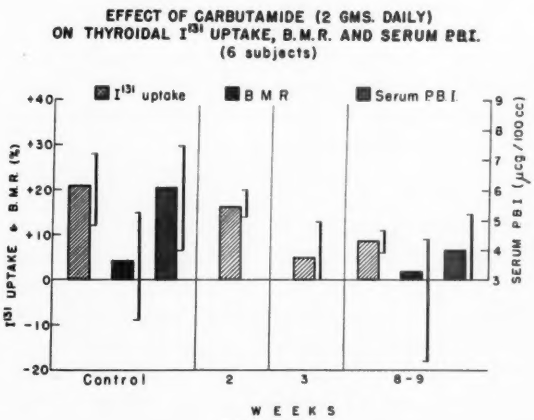

FIGURE 3

Patients receiving 1.0 gm. daily. The average pretreatment thyroidal uptake of radioiodine for this group of ten subjects was 23.0 per cent with a range from 11 to 38 per cent (figure 2). During the second and third weeks of treatment all of these subjects were

retested for thyroidal iodine uptake. The average value twenty-four hours after radioiodine was administered was again 23.0 per cent of the administered dose with a range from 12 to 36 per cent. Similar determinations at the end of nine and twenty weeks, respectively, yielded average values of 20.2 and 21.8 per cent respectively, with ranges of 12 to 24 per cent for the ninth week and 12 to 28 per cent for the twentieth week. Inasmuch as the standard deviation for the individual determinations was calculated at $\pm$ 4.3, none of the differences observed in figure 3 was statistically significant.

Patients receiving 2.0 gm. daily. In order to gain optimal control of the diabetic state in some of our patients, it was necessary to increase the dose of carbutamide to 2.0 gm. daily. Radioiodine pickup in six such subjects was determined before, at two or three and at eight or nine weeks following the initiation of therapy (figure 3). The average pretreatment twenty-four hour thyroidal uptake of radioiodine was 21.6 per cent with a range from 12 to 29 per cent. At two weeks, three subjects were tested and showed an average uptake of 16.3 per cent with a range from 14 to 20 per cent. The remaining three subjects were tested at the end of the third week and showed a twenty-four hour uptake of only 5.0 per cent with a range from 0 to 13 per cent. At the eight to nine week period of therapy the average twenty-four hour uptake of radioiodine by the thyroid was 8.7 per cent with a variation from 6 to 11 per cent. In view of the low thyroidal uptake it was decided not to continue the drug further, despite the fact that none of the subjects thus treated seemed to show any clear-cut clinical evidence of hypothyroidism.

B. *Influence of carbutamide on serum protein-bound iodine and basal metabolism.*

The averaged values for serum protein-bound iodine before and towards the end of twenty weeks of treatment of ten diabetic patients with 1 gm. of carbutamide daily were 6.4 and 6.8 μg. per 100 cc., respectively, with ranges from 4.2 to 8.4 and 4.0 to 9.0, respectively (figure 3). Corresponding averages for the basal metabolic rates were + 5.1 and + 7.4 per cent, respectively, with ranges of — 12 to + 18 and — 8 to + 17, respectively (figure 3). None of these changes has any statistical significance.

In six subjects treated with 2.0 gm. of carbutamide daily for nine weeks, the pretreatment value for serum protein-bound iodine varied from 4.0 to 7.5 with an average of 6.1 μg. per 100 cc., while values obtained during the ninth week of therapy ranged from 3.0 to 5.2 with an average of 4.8 μg. per 100 cc. These figures show a downward trend but with the exception of one patient, no value lay below the recognized limits of normal (4.0 to 8.0 μg. per 100 cc. in our laboratory). In these six subjects, the average pretreatment basal metabolic rate was + 4.3 per cent with a range from — 9 to + 15 per cent, and the post-treatment average was + 1.8 per cent with a range from — 18 to + 9 per cent. Here, too, there was a slight downward trend with two individual determinations below — 10 at the end of treatment with carbutamide, but the variations were wide, the subjects few and no statistically significant differences were present.

SUMMARY AND CONCLUSIONS

1. A single dose of 4 gm. of carbutamide may, but certainly does not always, depress the uptake gradient of the thyroid for tracer doses of I^{131}.

2. Four grams of carbutamide given daily for four days to diabetic subjects reduced the radioiodine uptake by the thyroid to approximately 25 per cent of the control value. Three grams of the drug daily for four days was associated with a reduction in radioiodine uptake to approximately 47 per cent of the control value.

3. Older patients given 1.0 gm. of carbutamide daily for twenty weeks showed no significant alteration in the values for radioiodine uptake by the thyroid, basal metabolism, or serum protein-bound iodine.

4. Older patients given 2.0 gm. of carbutamide daily for nine weeks developed a marked depression of the radioiodine uptake by the thyroid, and a tendency for a lower serum protein-bound iodine and a decreased basal metabolic rate.

5. It is concluded that the long-term use of carbutamide in doses of 2.0 gm. or more is associated with alterations in thyroid function, although at that level of dosage for periods up to nine weeks, older subjects do not show any clear-cut evidence of hypothyroidism.

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A Controlled Trial of Carbutamide in Diabetic Outpatients

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At the last meeting here in Indianapolis in March 1956, Dr. Joiner described the results of administering carbutamide to a series of hospitalized diabetic patients under controlled conditions. Since that time, we have conducted a trial of carbutamide on a group of outpatients from the Diabetic Clinic at the Pennsylvania Hospital. At present, thirty-nine patients have completed the trial and results on these patients are here presented.

MATERIAL

All patients were selected either because they were badly controlled by conventional means or because they were taking insulin in excess of 100 units daily or both. Over sixty patients have been included in the trial but several defaulted at some time during the course and the results of two others were discarded as unreliable. Eight patients are still under study.

METHODS

The patients were observed for three consecutive periods of two weeks each. Placebo tablets were given in weeks one and two and five and six. Carbutamide (0.5 gm. tablets) was given in weeks three and four. The patients were instructed to take six tablets on the first day, four on the second, and two on subsequent days of each study period. In this way, a loading dose was given in weeks three and four without arousing the patients' suspicion of a change in regimen. Each patient was seen by the dietitian at the beginning of the trial and at completion when the dietary intake for the previous six weeks was reviewed. If the patient was taking insulin, the dose was not changed during the trial period. Each patient was seen weekly when he was weighed and a fasting blood sugar sample drawn. Each was taught to test for glycosuria five times daily at prescribed intervals using Tes-Tape (Urine Sugar Test Tape, Lilly). All used tapes were dried, saved and submitted each week, and in addition each patient was asked to record the results of urine tests in a notebook provided for that purpose. The collected tapes were graded according to color without knowledge of the patient's identity. In this way, the frequency of occurrences of concentrations of 0, 0.1, 0.25, 0.5, and 2.0 (or more) percentages of glucose in the urine was estimated and designated as 1-5. Tes-Tape scores for the second week of each successive trial period were compared by the median and chi squared tests. A significant difference in glucose excretion was assumed to have occurred if the p value was less than 0.05.

After the completion of the six-week trial, all patients, whether showing a successful response to carbutamide or not, were asked to continue in the study. Carbutamide, 1.0 gm. daily, was given and the patient was asked to test his urine during the last week in every four, or more often, if it seemed appropriate. Insulin dosage was reduced if this could be done without increasing glycosuria, but no systematic attempt was made to eliminate insulin. Weight was recorded and blood sugar determinations made at each attendance. A white blood count was made at monthly intervals or more often if indicated.

Sugar determinations were made by Nelson's (1944) modification of Somogyi's method.

RESULTS

Analysis of the Tes-Tape scores allowed each patient to be placed in one of three groups—success, progressive success or failure. A successful response was taken to be a reduction in glucose excretion in week four, the second week of carbutamide administration, as compared with weeks two and six, the control periods. Further, there could be no significant difference in the scores between weeks two and six when these were compared. Six of the 39 patients, or 15 per cent, met these criteria and were considered successes.

In nineteen patients, or 50 per cent, week four showed significantly less glycosuria than week two, but in each case week six was also significantly better than week two. Because these diabetics showed continuing improvement during the entire course of the trial, they are listed as progressive successes. This result may have been due to better observation of diet and the general diabetic regimen rather than to the effect of carbutamide but it is worth noting that seventeen of the nineteen patients gained weight between weeks two and six while

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only two lost weight: one, 1 lb., the other, 3.5 lb. Therefore, it seems likely that some factor other than diet alone was operating.

The remaining fourteen patients, or 35 per cent, were classified as failures because there was no significant change in glycosuria either before, during or after the administration of carbutamide.

In general, the blood sugar determinations tended to parallel the results obtained from the Tes-Tape scores, but the number of individual determinations was small, two only for each period, and the scatter of the results was wide, particularly in those patients taking large doses of insulin. Of the fourteen failures, there were nine who might have been classified as successes or progressive successes on the basis of blood sugar values alone, but who were unequivocal failures when judged by the Tes-Tape scores. In the face of this, we do not feel that a weekly fasting blood sugar is very helpful in judging a response to carbutamide.

Patients have been followed from one to four months after the trial, but the results are necessarily incomplete. However, we are anxious to know whether the original trial period was accurate in predicting the subsequent course of these patients when continued on carbutamide. The results for each group up until the present time are as follows:

Of the six patients initially classified as successes, five have continued to show a response to carbutamide. Four of these five patients were taking insulin when the follow-up period started, an average of 40 units daily, and all four have been taken off insulin without an increase in glycosuria. All five who have improved have shown a gain in weight varying from 1-6 lb. The single patient who did not show a continuing response was a thin, labile female diabetic who was hospitalized during the follow-up period for a gynecologic operation and there lost weight. Carbutamide was stopped during this interval.

Fifteen of the nineteen progressive successes have been followed. Of these, ten have shown a continuing response to carbutamide and three have not. One patient was dropped because of the appearance of a mild exfoliative dermatitis which cleared when carbutamide was withdrawn, and in one patient, no clear-cut pattern is evident. All ten patients, who have improved, have gained weight, whether on insulin or not, whereas the three patients who failed to respond have lost weight. Six patients who were taking insulin at the completion of the trial have had the dose reduced or eliminated entirely. Of the three who did not show a response, two were on large doses of insulin—55 and 105 units per day respectively, and in neither has it been possible to reduce the insulin dose nor has there been any improvement in control while on carbutamide.

In the group of fourteen failures, ten have been available for follow-up study. Of these, seven are approximately the same now as they were in week six, while three appear to have shown improvement though this has not been verified statistically. In some instances, there has been a moderate reduction in daily insulin dose, but no patient who originally required insulin and who was also classified as a failure has been able to eliminate insulin from his regimen.

The number of patients in each group is too small to permit any detailed analysis of the other factors which are said to indicate whether a diabetic patient will respond to carbutamide. In this study, those patients who were classed as successes or progressive successes were usually older when their diabetes was discovered, and had often had the disease a shorter period of time than those who were failures. The successes tended also to be more obese and require less insulin. Yet there were many individual exceptions to this in all groups and it was not possible to predict in advance what the result of the trial would be in an individual patient.

SUMMARY AND CONCLUSIONS

The evaluation of patient response to hypoglycemic agents when controls are not used may be difficult and misleading. We feel that the method described here is a useful one for patients who have glycosuria because it permits controlled observations to be made and because the results may be analyzed statistically. The many uncontrolled variable factors may be presumed to operate equally throughout the six weeks of the trial and during the follow-up period. The method has objectivity, convenience and practicality in its favor.

Three patterns of response are demonstrated: unequivocal success, unequivocal failure and progressive improvement which persists for as long as two weeks following withdrawal of carbutamide. Patients classified initially as successes continued to show a response to the drug during the follow-up period. Those classed as failures during the trial period rarely showed improvement when carbutamide was continued. The progressive successes represented an intermediate group who generally improved during the follow-up period but not as dramatically as the outright successes. Almost all patients who had a significant reduction in glycosuria while on carbutamide gained weight during both the trial and follow-up periods. A single instance of toxicity, an exfoliative dermatitis, was noted during this study.

Final Discussion

DR. DUNCAN: We have used two methods in selecting patients who would probably respond to long-term therapy; one is about the same as the one Dr. Root used except that we gave a twelfth of the total daily diet at two-hour intervals during the time the curve was obtained. I think this made it a more severe test than if the patient were fasting. A second method was used in the patient on no insulin or small doses of insulin. Blood sugars were taken before each meal and at bedtime as a control curve and then similar tests the following day after 2 gm. of the drug before each meal and at bedtime.

On one slide of Dr. Root's the statement was that 95 per cent had good results. I take it that those were in the patients who had shown a favorable response to the test. The 69 per cent that had a satisfactory result on the other curve was the over-all result. The third question: Were those two patients who had hypoglycemic reactions taking any insulin at all at the time? We have seen hypoglycemic reactions only in patients who were taking insulin along with the drug, even though the dose of insulin was much less than they had previously needed.

DR. HOWARD ROOT: The figure 95 per cent is correct. . . That particular response test was reliable in 95 per cent. The over-all, as you said, was 69 per cent.

The two patients who had serious hypoglycemia did not have any insulin at the time nor for several days when hypoglycemia occurred. One of them died less than a week after he had hypoglycemia. He did not have hypoglycemia at death nor on the day before death, but he had had hypoglycemia on two separate occasions surely due to BZ-55 and not to insulin within a few days before he died.

DR. DUNCAN: That would not have been an hepatic death, would it?

DR. ROOT: No, nothing suggested hepatic death. It was simply one of those for which we have no explanation. Unfortunately we did not have a post-mortem examination. May I take this occasion to clarify one thing because I was apparently not clear about the hepatic death. You will remember that I mentioned the fact that this patient developed an acute hemolytic state. Some of you may have thought that the patient entered the hospital with jaundice due to hemolysis. That is not the case. The patient entered the hospital with no suggestion whatever of any hemolytic process. The patient entered the hospital with plain jaundice, with a high phosphatase, and this acute leukemoid state and an acute hemolytic phase occurred in the last 48 to 72 hours of life and was interpreted in the words of Dr. Sear as one of the phenomena which have been observed before in cases of acute liver disease with necrosis. I want to leave no doubt in anybody's mind that this patient entered the hospital without hemolytic jaundice.

DR. IZZO: We have had three patients who have developed hypoglycemic reactions on carbutamide alone. The most serious hypoglycemia was in the patient number three that I showed yesterday. She left the hospital on 2 gm. of carbutamide. At the end of a couple of weeks she reported severe reactions. We reduced the dose to 1.5 gm. but she still continued to have some reaction. At 1 gm. the blood sugars and urines are normal. Another patient on 1 gm. a day had reactions. We cut her to 0.5 gm.—still having trouble—and this patient now takes a half a tablet per day (0.25 gm.). The third patient at 1.5 gm. was having trouble but at 1 gm. did not have hypoglycemic reactions.

DR. RICKETTS: I believe I understood Dr. Root to say that he observed insulin potentiation in some juvenile diabetics.

DR. ROOT: That's correct, Dr. Ricketts.

DR. RICKETTS: I would like to quarrel a little with the use of that term. I don't believe one can speak legitimately of insulin potentiation as opposed to the stimulation of insulin release in any patient who has an intact pancreas, because you don't know whether the effect on reduction of insulin dosage is actually due to increased effectiveness of the administered insulin or to an increased release of insulin from the pancreas. I'd like to confine the use of the term insulin potentiation to circumstances in which there is no endogenous insulin present.

DR. CONN: We have had the same experience as Dr. Craig in a totally depancreatized patient. No effect of the drug was obtained which could be interpreted as potentiating administered insulin. It seems to me that there is very little good evidence to indicate that Orinase increases the peripheral utilization of carbohydrate.

DR. PECK: Do you include both compounds in that statement?

DR. CONN: Yes. It appears that the insulinase theory, at least with physiologically active amounts of these substances, is out of the picture. Until we have evidence for increased insulin levels in the blood, I personally would question that increased secretory activity of the pancreas is an action of these drugs. On the other hand

I think most of the evidence is highly suggestive that the major activity resides on those mechanisms which control the release of glucose from the liver in the fasting state.

We, and also Dr. Thorn's group, have done a number of experiments which show that the level or amount of adrenal steroids in blood and urine is not altered by these compounds. Although there is no evidence that these compounds alter or suppress adrenal cortical function, Dr. Lukens' paper and other reports cited yesterday show that adrenalectomy makes these compounds much more effective in producing hypoglycemia. I'd like to know why adrenalectomy is so much more effective than hypophysectomy in increasing the sensitivity of the animal to these compounds. A hypophysectomized animal is capable of putting out minute amounts of adrenal cortical steroids and these may have important bearing on the hepatic aspects of the mechanism by which this compound is effective.

From a clinical point of view I think that Dr. Duncan has expressed my feelings, and I am in complete agreement with him.

DR. HOWARD ROOT: May I discuss what I meant by potentiation of insulin? This happens in children as well as adults who may be presumed still to have the capacity to increase their carbohydrate tolerance. Initially you give 3 gm. of carbutamide or tolbutamide as a test dose and the blood sugar shows zero effect. Now give the subject insulin alone in a small dose (three or four units) and the blood sugar goes down 10 per cent. Now give the same individual the same insulin dose along with carbutamide or tolbutamide and the blood sugar falls 40 to 50 per cent. Now, perhaps this is not potentiation of insulin. For we must recall in this connection that Dr. Houssay showed a curve in Brussels last summer demonstrating this effect in depancreatized animals in which insulin action was potentiated by BZ-55.

DR. RICKETTS: You still don't know for sure whether in your patients the pancreas has not kicked out a little more insulin.

DR. ROOT: I agree.

DR. LUKENS: Throughout all of these discussions, particularly throughout the clinical observations, there has been little discussion of the quantitative significance of the effect of these drugs upon the twenty-four-hour metabolism. The amount of glucose which disappears from the urine is on the average 10 to 30 gm. a day; the proportion of the available glucose of the diet that represents is approximately 10 per cent. Those diets are high by comparison with pre-insulin standards or with any regimen which controls a diabetic without insulin.

DR. KINSELL: To second Dr. Ricketts' remarks it would seem to me that there are many reasons to assume that any individual who responds significantly to the sulfonylureas has some capacity to make endogenous insulin. The two unstable juvenile diabetics that Dr. Talpers presented were studied under chemically constant conditions on the metabolic ward on purposely inadequate insulin dosage with purposeful measured urinary spills. The one receiving very large amounts of sulfonylurea had complete disappearance of glycosuria; the degree of response appeared to be somewhat proportional to the dose used. This could be construed to mean that it took a very large push to turn off some insulin inhibitor or to increase insulin production. The other boy with precisely the same program gave no response at all or if anything had an increased glycosuria. This could mean that he had no ability to make endogenous insulin.

I cite the case of the child who had a highly diabetic glucose tolerance but no symptoms. He was hospitalized and placed on a high-protein-high-fat-low-carbohydrate regimen. He had no significant increase in his over-all diabetic tendency. With the administration of relatively moderate amounts of sulfonamide he reverted repeatedly to normal glucose tolerance. On 0.5 gm. of BZ-55 a day he had hypoglycemic symptoms with low blood sugar values. He is receiving 0.25 gm. at the moment and we will probably reduce this further. We would construe this to mean that he still had highly viable beta cells which were still capable of responding to BZ-55.

DR. WILLIAMS: In connection with Dr. Conn's remarks on the adrenal, adrenal demedullation alone will greatly increase sensitivity to these compounds although not to the extent that a total adrenalectomy will.

I want to comment on some practical considerations when following patients for possible toxic reactions. I think that it is important to avoid relying with too much conviction on *routine* white counts. I have had considerable experience along this line back in the days of the different anti-thyroid compounds. We used to do an enormous number of white counts, but one can find an absolutely normal white count on a given day and a normal differential and two or three days later you have frank agranulocytosis. Now, having done these routine counts, if the patient says, "Doctor, I don't feel well," you are apt to say to yourself, "Well, I examined Mrs. So-and-So three days ago. It couldn't be a fall in white blood count," when in actuality that might be it. By all means get that white count and see what the situation is.

DR. McGAVACK: We have also seen the reverse of

what Dr. Williams mentioned. We have seen a normal total white count, granulocytes going as low as 25 per cent, reverting to normal within six to eight hours without any other change and with no symptoms on the part of the patient. The patient is able to continue the drug without having any trouble. So I think it is totally unreliable, and I believe Barr's original work, taking counts every twelve hours, showed that he was no better able to pick up these episodes than the people who didn't take them at all.

But I want to ask two questions. Can Dr. Kirtley give us any information on the dosage and duration of treatment with the sulfonylureas in relation to the side effects produced? It seems to me that would be very important in long-term treatment.

Another question to Dr. Field: Did his patient continue her bacchanalian fervor right up to the end? It seems to me that as a rule Bacchus and sulfonamides don't go very well together.

DR. PECK: Before Dr. Kirtley speaks, in connection with Dr. Williams' remarks about white count, I'm not sure how one might handle that situation in connection with the usual requirements of the Food and Drug Administration who of course are primarily interested in the safety of any compound and in setting out regulations to insure due and proper warnings.

DR. KIRTLEY: We haven't had time to study completely the whole data. So we don't know too much about the total duration of administration in all cases. However, we do have the impression that very few have developed past thirty days of administration. It seems that if they go over thirty days they are all right, have "gotten over the hump," and they have no further reactions. Some instances of course are reported as nausea, vomiting, headache, and so on for a period of a day or so—the minor symptoms. Most of the deaths reported have occurred about thirty days after the administration of the initial dose. It seems that some time between ten days and thirty days is when the majority of the side reactions are going to appear.

If you will recall our first two conferences, when we were still trying to establish positive effect, we recommended a dose far higher than was necessary, namely up to 6 gm. a day. I think that some of us continued using that higher dose level. However, since March or April, we have advised that the first dose should be a loading dose—2.5 gm. the first day, 1.5 gm. the second, and then 1 gm. each day thereafter. I think most of the investigators have followed that schedule. We have indicated that if 3 gm. did not produce results, treatment was a waste of time. I think very few have gone over that level and most patients are getting around 1 gm. a day, some 1.5 gm., and perhaps 2.0 gm. maximum.

DR. WILLIAMS: There is a question I'd like to ask Dr. Kirtley. He grouped together agranulocytosis and leucopenia. It makes a great difference which you have; leucopenia can mean nothing of any real consequence to the patient, where agranulocytosis is highly significant. Can you give us figures?

DR. KIRTLEY: No, I can not give you a breakdown, because this is just the information we got from the short report forms. They simply report a lowering in the blood count. They may have been looking only for granulocytes or they may have taken the total white count. I think in the majority of cases they are simply taking a total count. We have had some reports in which there has not been much alteration in the total count with almost a disappearance of granulocytes.

DR. PECK: Those compilations are based on a short form report. This is not the long form which is set up for punch card statistical analysis, and will yield more complete information on individual cases.

DR. FIELD: The history of alcoholism in the patient that we reported had been denied by the patient but relatives were the ones who supplied the facts. We did get information from relatives that the night before she died she had been partaking of alcohol. However, as far as we know she had not had anything to drink on the day of death.

I would like to agree wholeheartedly with what Dr. Williams said about white counts in general. In our own case (and it's always easier to use the retrospectoscope than to realize what's going on at the time) during treatment she had developed 6 per cent eosinophilia when all her previous levels had never been higher than 2 per cent. Also there was a drop in her poly count from 43 per cent to 31 per cent. Had we been a little more acutely aware of the possibilities and stopped the drug, this patient might still be alive today.

DR. ANDERSON: You recall that during the thiouracil period it was the cessation of therapy and then its resumption that seemed to bring out acute symptomatology. I wonder whether this has been reported in the group sent in to Dr. Kirtley.

DR. KIRTLEY: There has been no indication of that.

DR. PECK: It has been a fairly common custom in Germany to treat people intermittently. Is that not true, Professor Achelis? (Yes). There the sensitivity reactions have been of quite low incidence.

DR. DUNCAN: The islets are inextricably mixed up with this reaction of the drug. Is it possible that the

islets produce another substance that alters the release of glucose from the liver, and that this substance may be removed in the manufacture of insulin? In other words, is there a definite difference between endogenous insulin and the exogenous insulin?

DR. PECK: That is a situation which everyone has speculated about. It's true of all other tissue extracts. They get pretty rough treatment and how one would determine what the hormones are like before they were given the chemical treatment has been a rather insoluble problem.

DR. DUNCAN: Dr. Haist told me of one example in which there is a difference between the native product and that which we use commercially.

DR. HAIST: The experiment to which I was referring concerned a pituitary fraction that was reported many years ago to impart resistance to insulin when given by injection but was not diabetogenic. That would make one think of the possibility that exogenous insulin was reacting differently from endogenous insulin.

DR. IZZO: I am greatly disturbed because there is something here that is paradoxical. If the sulfonylureas stimulate the pancreas to put out more insulin, then we should see the peripheral effects of insulin. As Dr. Conn has shown this morning, and as others report, there is no evidence to show that there is increase in peripheral insulin action. Now, if these drugs stimulate insulin secretion, what kind of insulin is being stimulated? If it isn't stimulating insulin secretion, what kind of effect is it having? It seems to me that if it is not stimulating insulin secretion, then it should have some effect upon the liver and if you give insulin to a depancreatized human there should be some response to sulfonylureas, but this has not been observed. There is the excellent experiment of Miller and Dulin (Science *123*: 584, 1956). They gave Orinase to an experimental animal and found an increase of liver glycogen and no increase in muscle glycogen. But insulin produced an increase in muscle glycogen and little or no change in liver glycogen. This leaves me very confused as to what actually is happening. The stimulation of insulin secretion cannot possibly be the explanation of the principal action of the sulfonylureas.

I would just like to add one further comment. If the reaction to the drug is of a small order, the methods we have used may have been too crude to detect these small changes. There has been some suggestion of that, for instance in sulfonylurea inhibition of glucagon hyperglycemia. For instance, the effect is small and if one uses a large amount of glucagon, one can overwhelm the effect of the drug and fail to demonstrate it. Maybe if we refine our technics we might see these differences.

PROFESSOR ACHELIS: I am very impressed by the case reported by Dr. Field, and I think there is a high probability that this death was caused by the treatment. But, speaking frankly, I have to say that we in Germany never took the risk of such a high dosage in treating diabetes. We must remember that the BZ-55 is a depot or accumulating drug. Fifty per cent of a single dose is excreted only after a period of forty-four hours. If you give the next dose after twenty-four hours, there is a considerable residue and one can figure out that a repeated 4 gm. dose is equivalent to a single dose of 16 gm. One would have to be very cautious, I think, when repeating high doses for several days. While trying the compound as an antibacterial agent three years ago, with the high continued doses mentioned, we observed toxic reactions like malaise in a certain number of cases.

DR. PECK: Our experience was similar. Dr. Kirtley mentioned something about that this morning. In relation to the long-term effect of BZ would you want to say anything in that connection regarding the other compound, which does have a difference in solubility?

PROFESSOR ACHELIS: I think that there is quite a difference. If you diagram the effect it is roughly similar to comparing the quick action of insulin with the slow action of protamine insulin. The duration of blood sugar lowering by Orinase is about seven hours, for carbutamide about forty-four hours. We have here a quick acting and a slow acting drug, and therefore, we cannot compare the dosages gram for gram.

DR. HOWARD ROOT: Are there well-known differences in the absorption or excretion of BZ-55?

PROFESSOR ACHELIS: There are individual differences. The absorption of BZ-55 is very quick. If you compare the peak blood level of the drug after intravenous or oral administration there is only a small difference between the two. Therefore, we concluded that the absorption must occur very quickly.

DR. HOWARD ROOT: We have become more and more confirmed in the opinion that if we cannot control any patient on 1 gm. per day, we will not continue him on the drug. I have a feeling that it is unsafe to continue and carry any diabetic patient for any long period of time on more than 1 gm. a day.

PROFESSOR ACHELIS: I think this is the first reason for the impressive difference between German and the American findings. We use smaller dosages. In the second place it may be that the sensitivity for allergic reaction is greater in the United States. We have compared many compounds coming from American firms

with a reported incidence of 4 per cent of allergic reactions. We gave it in our clinics and found 1 per cent.

DR. HOWARD ROOT: Has anybody commented on the significance of the statement which appeared many years ago, that allergic reactions are related to the occurrence of the NH_2 groups?

PROFESSOR ACHELIS: No. You can have reactions in compounds completely aliphatic and without NH_2 groups.

DR. RICE: I have some data on the effects of the sulfonylureas on the catabolism of glucose. At our suggestion, Dr. Tolbert of the Radiation Laboratory of the University of California has carried on some experiments in which he has measured the C^{14}-Co_2 in the respiratory air from rats which have been given uniformly U-C^{14}-glucose. These animals were either fasted normal or alloxan-diabetic animals. I want to summarize these rather limited data. They are also available in the University of California Radiation Laboratory Report Series, No. UCRL 3503.

The data give the production of C^{14}-Co_2. In normal fasted animals about 5 per cent of the intravenously administered U-C^{14}-glucose appears in the respiratory carbon dioxide in forty minutes, and approximately 28 per cent in two hours. When insulin is given one hour prior to the labeled glucose, the total C-^{14}-carbon dioxide appearing in forty minutes is doubled. In two hours the value (28 per cent) obtained is about the same as in the control animals. When Orinase is given, I think that we can say that there is no significant difference in the U-C^{14}-glucose burned in a forty-minute interval to C^{14}-Co_2. It would appear in the case of carbutamide that approximately the same amount of glucose is burned to carbon dioxide in a two-hour period as in the case of control animals or animals treated with insulin. In the case of Orinase the value is somewhat higher. We observed an average (four animals) of about 37 per cent.

The same experiments have been run using U-C^{14}-fructose as a control, and in a forty-minute period the labeled carbon dioxide recovered in the respiratory air is essentially the same as that obtained in the case when labeled glucose is used (4 to 8 per cent) in the control animals, animals treated with insulin, or with either of the two sulfonylureas. At two hours, the amount of the U-C^{14}-fructose oxidized to C^{14}-Co_2 ranges from 22 to 29 per cent in the four categories; that is, control animals, untreated, insulin treated, and those treated with the two sulfonylureas. There appears to be no distinction in the case of fructose, in the four situations.

When labeled acetate was used approximately 15 to 26 per cent was converted to carbon dioxide in a forty-minute interval. The control animals, that is, those having no treatment, gave the higher values; the carbutamide treated animals gave the lowest value. Two hour values are: control animals, 54 per cent; insulin treated, 51 per cent; carbutamide treated, 44 per cent.

I would like to refer to some data on the effect in an alloxan-diabetic animal of these agents. We have two situations. In one instance, the sulfonylurea is given approximately two to three weeks after alloxan treatment. In diabetic animals we get a curve of accumulation of C^{14}-Co_2 lower than that obtained with normal animals. If one day after the measurement of the control curve the animal is given orally Orinase (1 gm. per kg.), we get a very slight enhancement of Co_2 production. The following day after another 1 gm. per kg., the value goes up to close to that of the normal animal. The next day it is down somewhat, and on the following day, when the experiment is repeated in the absence of Orinase, we get a diabetic curve again. If this alloxan diabetic animal is given insulin, the curve is essentially that of the normal animal.

The next situation I want to describe is the same general series of tests run about forty-five days after the administration of alloxan. The diabetic animal given insulin gives a Co_2 accumulation similar to that of the normal animal. If Orinase is now given we obtain typical diabetic curves on the first and also the second day. The same results are obtained with carbutamide. It appears from the data that it is impossible for either of these two sulfonylureas to give an insulin-like effect. These results are distinctly different from the situation that we have at two to three weeks, after alloxanization. In other words it appears that the later animals are not completely diabetic.

The third thing that I think should be mentioned is an experiment in which Orinase and insulin are given together in the following manner. Orinase is given on the first day, the next day it is given again, one hour later insulin is injected, and immediately this is followed by labeled glucose. The maximum activity in the control animals where Orinase is not given is reached about four to eight hours following the administration of the insulin and the glucose. When Orinase is also given with insulin, the same total duration of action and the same period of maximum activity for the insulin was observed.

DR. GOLDNER: I should like to come back to the incidence of side reactions and particularly fatalities in Germany. The question about autopsy findings has been posed to Professor Achelis several times. In searching the

German literature I have found reports of eight or nine deaths which occurred during sulfonylurea medication. The autopsy reports, however, are incomplete; they mention intercurrent or complicating diseases which were the cause of death but give histologic findings only in regard to the pancreas and the cellularity of the islets. Ferner and Runge* thus report on three deaths which, as I believe, occurred in Bertram's series in Hamburg. The early cases in Germany were treated with rather high doses of BZ-55, 3 to 6 gm. per day during the first few days and 1 to 2 gm. from there on. I wonder whether Professor Achelis has information about the complete autopsy findings and particularly whether signs of hypersensitivity reactions were found anywhere in the vascular system. A second series was reported by Creutzfeldt.† It refers to, as I believe, six cases, treated mainly with D 860. Here too only the histologic findings in the pancreas are described and again complicating diseases such as carcinoma, cerebral vascular accident, coronary infarct, or cirrhosis of the liver are given as cause of death. Other organ—and particularly vascular—findings are not mentioned. In none of these eight or nine cases were there any significant alpha-cell changes.

In view of the hypoglycemic reactions which Dr. Root and Dr. Izzo mentioned, it might be worthwhile to remember that severe and prolonged hypoglycemia and two deaths were reported after IPTD in 1942 from Janbon's Clinic in Montpellier.‡ These two hypoglycemic deaths were probably responsible for the fact that this drug which is closely related to the sulfonylureas was not tested clinically as an antidiabetic agent.

PROFESSOR ACHELIS: We also have two cases of prolonged severe hypoglycemic reactions with BZ-55 alone, not combined with insulin. They were not fatal but it was very difficult to restore this patient to a normal blood sugar level. I think I can say that in all except one, autopsy was complete. This was the first; the alpha cells were reported to be destroyed. In the next investigation by Ferner, there was no damage to the alpha cells, but I don't know whether autopsy was complete.

DR. WILDBERGER: In regard to Dr. Field's case it was suggested that excessive dosage had been given. I think you said that the BZ-55 blood level was only 13.6 mg. per cent the day before death. Is that right?

DR. FIELD: Yes.

DR. WILDBERGER: That is within therapeutic range. I wonder what 4 gm./day could have to do with the death. We achieved those levels with 1.5 gm./day.

DR. FIELD: We did blood levels every day and they were never greater than 15 mg. per cent. The 4 gm. was given in divided doses. In retrospect it would be considered an excessive dose. At the time it was given other people were using this dose and since we did not have what we considered a satisfactory response at 2.5 gm. we increased it to 4 gm. I would also like to point out that when French in the *American Journal of Pathology* in 1946, reviewed cases of interstitial myocarditis which were attributed to sulfonamide hypersensitivity, there was not any particular correlation between the amount of sulfonamide received and the hypersensitivity.

DR. MARY ROOT: Although one has to be cautious in extrapolating from animals to man, I think one thing that might be pointed here is that in a few experiments I have given carbutamide to rabbits orally and measured not only their blood sugar response but also the blood levels of carbutamide. A dose in one rabbit that will give perhaps a 10, 15, or even 20 mg. per cent level in the blood, will in another animal give a practically zero level in the blood. I wonder if perhaps that isn't the same thing that may be going on in patients and therefore blood levels are going to give you better information than the actual dose administered.

DR. KRAHL: I was asked by Dr. Rice to comment on his data on $C^{14}O_2$. Studies with labeled glucose of this sort, on first sight seem to suggest an increase in peripheral utilization in the rat caused by BZ-55. Such studies would be very helpful to reveal mechanisms of action and can be carried out with very small doses of C^{14} even in patients. But it would seem to me to be useful to use not a single dose of the labeled glucose but to infuse the isotope at a constant rate so as to establish a flat base line for the CO_2 excretion upon which any drug effect can be more easily evaluated.

DR. CONN: I would like to ask Dr. Krahl what data suggest that there is increased peripheral utilization due to the sulfonylurea compounds. In the whole animal is there any increase of $C^{14}O_2$ from U-C^{14} glucose produced directly or indirectly by the drug? The other point I wish to make about this is that you can't really draw the conclusion with certainty because if the drug is suppressing glucose output, then the single tracer dose of glucose that is given is diluted by a smaller pool than in the control animal. The specific activity in the blood is therefore higher and apparently you would get more expired labeled CO_2. Therefore, you must start by having a flat base line.

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* Ferner, H., and Runge, W.: Deutsche med. Wchnschr. *81*: 331-32, 1956.

† Creutzfeldt, W.: Deutsche med. Wchnschr. *81*:841, 1956.

‡ Janbon, M., Lazerges, P., and Metropolitanski, J. H.: Montpellier Med. *21/22*:489, 1942.

Present State of Knowledge Concerning Effects of the Sulfonylurea Compounds in Diabetes Mellitus

Report of the Committee on Summarization of the Conference

PHYSIOLOGIC AND BIOCHEMICAL ASPECTS

1. *Peripheral Tissues.* No convincing evidence has been adduced that the sulfonylurea compounds increase utilization of glucose by peripheral tissues: They do not lower the blood sugar of eviscerated dogs (Levine), rabbits (Wick) or rats (Ingle) with or without supplementary insulin; they do not increase glucose uptake or glycogen deposition by isolated rat diaphragm, or glucose uptake by rat adipose tissue, in a medium of physiologic salt solution (Krebs-Ringer) where insulin has a pronounced effect (Krahl, Cahill, Clarke: *Canad. M.A.J.*, 1956, and Field); and they do not increase the arteriovenous blood sugar difference after glucose loading (Goldner, Volk and co-workers).

2. *Liver.* Studies of the effects of these substances on liver glycogen levels have given conflicting results, possibly because of differences in experimental conditions such as lack of uniformity in species, nutritional state, timing of samples, and duration and intensity of treatment.

There is evidence that the sulfonylurea compounds inhibit the output of glucose from the intact liver (Anderson, Bondy), especially that derived from fructose and galactose (Renold, Craig) and possibly that resulting from stimulation by glucagon and epinephrine (Izzo; not confirmed by Fajans, Anderson, Goldner). On the other hand, in in vitro experiments with liver slices from normal fasted and fed rats, addition of these compounds to the medium did not affect glycogenolysis or glucose output (Cahill, Sutherland). The ordinarily glycogenolytic effect of glucagon and epinephrine in such slices appears to be inhibited by the drugs (Vaughan). Chronic medication of normal rats was followed by a reduction in glucose-6-phosphatase of the liver (Cahill, Haist, Kuether), but this effect was in no instance correlated temporally with the hypoglycemia. It was not observed in the livers of alloxan diabetic rats. Destruction of insulin in either the whole animal or isolated liver preparations was not significantly reduced by concentrations of the drugs sufficient to cause hypoglycemia (Vaughan, Williams).

3. *Pancreas.* The great preponderance of evidence indicates that these sulfonylurea compounds have no effect on the alpha cells. While toxic doses resulted in a distinct decrease in the insulin content of the pancreas (dogs), so-called therapeutic doses over long periods of time had no such effect (M. Root).

In rats, administration of carbutamide caused an increase in weight of islet tissue (Haist). In dogs, infusion of a solution of carbutamide into the arterial supply of the pancreas in amounts insufficient to produce "therapeutic" levels in the peripheral blood resulted in an approximately 20 per cent fall in peripheral blood glucose (A. and J. Colwell).

In discussion, reference was made to the work of Foa (*Fed. Proc.*, 1956, in press), who found in cross-circulation experiments in dogs that blood from the pancreaticoduodenal vein, but not the mesenteric vein, of the donor dog treated with carbutamide caused a decline of blood sugar in the recipient.

These observations suggest a stimulating effect on the beta cells. However, the failure of the drugs to alter the rate of blood glucose disappearance during glucose tolerance tests in normal and mildly diabetic subjects (Fajans, Renold) is inconsistent with this hypothesis. The question was raised of whether the sulfonylurea drugs might overstimulate and finally exhaust the already inadequate islet system of the diabetic patient. In this connection, it was reported that one partially depancreatized rat with sugar-free urine developed glycosuria when treated with carbutamide, the excretion of glucose ceasing upon withdrawal of the drug. (Haist)

There is agreement that carbutamide and tolbutamide have no effect on the blood sugar in the complete absence of insulin (Achelis, Lukens, Levine and others). The question of whether insulin action is potentiated by these preparations cannot be satisfactorily answered by the clinical observations or animal experiments reported to date (Achelis, M. Root, Fajans, Cahill, Campbell; [*Canad. M.A.J.*, 1956]). The contrast between insulin and the sulfonylureas in their effect on blood pyruvate (Fajans) reveals a biochemical distinction which should stimulate further study.

The adrenalectomized animal (dog, cat, rat) treated with sulfonylureas showed an exaggerated hypoglycemic response, whereas the hypophysectomized animal did not (Lukens, Williams, Goldner, Volk et al., Houssay [quoted in discussion]). Thus, the response of such

animals to these drugs is different from their response to insulin. The failure of hypophysectomized animals to display extreme hypoglycemia after administration of the drugs appears to be inconsistent with the previously cited studies suggesting stimulation of beta cell activity. This discrepancy might be resolved if more were known, in quantitative terms, about the responsive capacity of the beta cells in both the normal and the hypophysectomized organism.

4. *Thyroid.* Chronic administration of sulfonylurea compounds resulted in thyroid hyperplasia, at least in animals (Logothetopoulis, cited by Haist), and, with carbutamide, a decrease in uptake of radioiodine by the thyroid in man (McGavack, Kinsell).

5. *Pituitary and Adrenal Cortex.* Data thus far reported indicate that the drugs being studied have no measurable effect on the anterior hypophysis or the adrenal cortex of man or animals (Fajans, Renold, Kinsell, Izzo).

CLINICAL ASPECTS

1. *Field of Usefulness.* Previous experience indicating that the drugs are most effective in older, obese patients with mild diabetes of relatively short duration is confirmed. They have some hypoglycemic activity in exceptional cases of juvenile diabetes. Short-term tests of response to the drugs are moderately useful, but not infallible, in predicting a favorable result of maintenance therapy.

2. *Toxicity.* In one compilation of data involving 7,193 patients, 389 (5.36 per cent) experienced side reactions consisting chiefly of skin rashes, gastrointestinal disturbances, leukopenia and generalized allergy in the order given. In another study, the incidence of side effects among 279 cases was 9.2 per cent (H. Root). Well-marked hypoglycemic reactions were reported in several patients on drug therapy without injected insulin.

There were eight deaths during treatment, with two each due to "sulfonamide sensitivity" and liver involvement respectively (Kirtley). Five autopsy reports are known (Harris), of which that of Field showed extensive focal interstitial myocarditis similar to that seen in sulfonamide sensitivity. The death reported by Root featured hepatitis which pathologists distinguished from the viral forms and which was labeled toxic. Field's case also had focal granulomatous lesions of the liver, spleen and lymph nodes.

In all cases of liver damage, including a number with recovery, liver function tests were normal except for the van den Bergh in the presence of jaundice, and the serum alkaline phosphatase (Root). The latter test appears as an important one which should be used earlier in following treated patients. The possibility that eosinophilia might foretell an oncoming toxic reaction was suggested by Field. In discussion, Dr. Williams cited the experience with thiouracil as making blood counts unnecessary as a routine, and instruction and observation of the patient paramount in forestalling leukopenia.

These first American reports of toxicity make it clear that one may expect from these drugs not only minor allergic reactions such as dermatitis, but also severe generalized changes. Apparently 1 gm. per day is generally regarded as a safe maintenance dose, but even this is no guarantee against allergic sequelae, since these are unrelated to the level in the blood.

This symposium reflected a considerable increment in knowledge of the sulfonylureas, particularly with respect to delineating and delimiting possible mechanisms of action, as compared with the symposium of March 1956.

Areas which call for further investigation are: (a) The difference in blood pyruvate behavior when a sulfonylurea, as opposed to insulin, is given. (b) The greater sensitivity of adrenalectomized, as opposed to hypophysectomized, animals exposed to the drugs. (c) The effect of the drugs in depancreatized animals treated with insulin. (d) The question of whether insulin production or release is stimulated. (e) The effect of glucagon in the presence of sulfonylureas on glucose output by the liver. (f) The difference in hypoglycemic effect between oral and intravenous administration. (g) Further statistical studies of toxicity.

HENRY T. RICKETTS, M.D., *Chairman*
FRANCIS D. W. LUKENS, M. D.
M. E. KRAHL, PH.D.

Le Stato Presente del Cognoscentias Relative al Effectos del Compositos de Sulfonylurea in Diabete Mellite

Reporto del Committee pro le Summarisation del Resultatos del Conferentia

ASPECTOS PHYSIOLOGIC E BIOCHIMIC

1. *Texitos Peripheric.* Nulle datos convictive esseva adducite in supporto del these que le compositos de sulfonylurea augmenta le utilisation de glucosa per le texitos peripheric. Illos non reduce le sucro sanguinee in eviscerate canes (Levine), conilios (Wick), o rattos (Ingle) con o sin insulina supplementari. Illos non augmenta le acceptation de glucosa o le deposition de glycogeno per isolate diaphragmas de ratto o le acceptation de glucosa per texitos adipose de ratto in un medio de solution salin physiologic (Krebs-Ringer) ubi insulina ha un effecto pronunciate (Krahl, Cahill, Clarke: *Canad. M.A.J.,* 1956, e Field). E illos non augmenta le differentia arterio-venose de sucro post incargamento de glucosa (Goldner, Volk, e collaboratores).

2. *Hepate.* Le studio del effectos de iste substantias super le nivellos hepatic de glycogeno ha producite resultatos contradictori, possibilemente in consequentia de differente conditiones experimental.

Il existe observationes que indica que compositos de sulfonylurea inhibi le livration de glucosa per hepates intacte (Anderson, Bondy), specialmente in le caso de glucosa derivate ab fructosa e galactosa (Renold, Craig) e possibilemente in le caso de glucosa resultante del stimulation per glucagon e epinephrina (Izzo; non confirmate per Fajans, Anderson, Goldner). Del altere latere, in experimentos in vitro con sectiones de hepate ab rattos normal post periodos de jejuno o post alimentation regular, le addition de iste compositos al medio non afficeva le glycogenolyse o le production de glucosa (Cahill, Sutherland). Le glycogenolyse que es normalmente effectuate in tal sectiones per le presentia de glucagon e epinephrina es apparentemente inhibite per le drogas (Vaughan). In rattos normal le medication chronic esseva sequite per un reduction del glucosa-6-phosphatase del hepate (Cahill, Haist, Kuether), sed iste effecto monstrava in nulle caso ulle correlation temporal con le hypoglycemia. Illo non esseva observate in rattos alloxano-diabetic. Le destruction de insulina in animales intacte e etiam in isolate preparatos hepatic non esseva reducite de maniera significative per concentrationes del drogas que suffice a causar hypoglycemia (Vaughan, Williams).

3. *Pancreas.* Le plus grande portion del observationes indica que iste compositos de sulfonylurea ha nulle effecto super le cellulas alpha. Durante que doses toxic resultava in un distincte reduction del contento de insulina in le pancreas de canes, doses del genere designate como therapeutic produceva nulle tal effecto mesmo in le curso de prolongate periodos de tempore (M. Root).

In rattos le administration de carbutamido causava un augmento de peso del texito insular (Haist). In canes le infusion de carbutamido in le arterias afferente del pancreas, in quantitates non sufficiente a producer nivellos "therapeutic" in le sanguine peripheric, resultava in un reduction del nivello de glucosa in le sanguine peripheric per 20 pro cento (A. e J. Colwell).

In le discussion oral, mention esseva facite del labores de Foa (*Fed. Proc.,* 1956, sub pressa), qui trovava in experimentos de circulation cruciate in canes que sanguine ab le vena pancreaticoduodenal sed non ab le vena mesenteric de donatores tractate con carbutamido causava un reduction del sucro sanguinee in le recipientes.

Iste observationes suggere un effecto stimulative super le cellulas beta. Tamen, le facto que le drogas non altera le rapiditate del disparition de glucosa sanguinee in tests del toleration de glucosa in subjectos normal o levemente diabetic (Fajans, Renold) non es compatibile con iste hypothese. Esseva sublevate le question si le drogas sulfonylureal effectua possibilemente un stimulation excessive e finalmente le exhaustion del jam inadequate systema de insulas in le patiente diabetic. In iste connexion il esseva reportate que un partialmente dispancreatisate ratto con urina sin sucro disveloppava glycosuria quando illo esseva tractate con carbutamido e que le excretion de glucosa cessava quando le droga esseva discontinuate (Haist).

Le autores es de accordo que carbutamido e tolbutamido ha nulle effecto super le nivello del sucro sanguinee in le absentia complete de insulina (Achelis, Lukens, Levine, e alteros). Le question si le action de insulina es potentiate per iste preparatos non trova un responsa satisfactori super le base del observationes clinic e del experimentos animal usque nunc reportate (Achelis, M. Root, Fajans, Cahill, Campbell; [*Canad. M.A.J.,* 1956]). Le contrasto inter le effectos de insulina e le sulfonylureas super le pyruvato sanguinee (Fajans) revela un distinction biochimic que deberea

stimular studios additional.

Adrenalectomisate animales (canes, cattos, rattos) tractate con sulfonylureas exhibiva exaggerate responsas hypoglycemic. Isto non valeva in le caso de animales hypophysectomisate (Lukens, Williams, Goldner, Volk, et al., Houssay [citate in le curso del discussion]). Assi le responsa de tal animales a iste drogas differe ab lor responsa a insulina. Le facto que animales hypophysectomisate non exhibi extreme grados de hypoglycemia post le administration del drogas pare esser in disaccordo con le previemente citate studios que suggereva le occurrentia de un stimulation del activitate del cellulas beta. Iste discrepantia poterea possibilemente esser clarificate si plus extense informationes de character quantitative esseva disponibile relative al capacitate de responsa del parte del cellulas beta in le organismo normal e in le organismo hypophysectomisate.

4. *Thyroide.* Le administration chronic de compositos sulfonylureal resultava in hyperplasia thyroide—al minus in animales (Logothetopoulis, citate per Haist)—e, in le caso de carbutamido, in un reduction del acceptation de radioiodo per le thyroide human (McGavack, Kinsell).

5. *Glandula Pituitari e Cortice Adrenal.* Le datos usque nunc reportate indica que le drogas sub investigation ha nulle effecto mesurabile super le hypophyse o le cortice adrenal de homines o animales (Fajans, Renold, Kinsell, Izzo).

ASPECTOS CLINIC

1. *Campo de Utilitate.* Previe experientias ha indicate que le drogas ha lor plus alte grado de efficacia in patientes de etate plus avantiate qui es obese e ha leve grados de diabete de relativemente breve duration. Istos es confirmate. Le drogas ha un certe grado de activitate hypoglycemic in casos exceptional de diabete juvenil.

2. *Toxicitate.* In un compilation de datos relative a 7.193 patientes, il esseva notate que 389 casos (5,36 pro cento) exhibiva reactiones lateral consistente principalmente de eruptiones cutanee, disturbationes gastrointestinal, leucopenia, e allergia generalisate (in iste ordine). In un altere studio, le incidentia de effectos lateral inter 279 casos amontava a 9,2 pro cento (H. Root). Distincte reactiones hypoglycemic esseva reportate in plure patientes qui recipeva le drogas sin injectiones de insulina.

Occurreva octo mortes durante le tractamento. Duo esseva le resultato de "sensibilitate sulfonamidic"; in duo alteres le causa esseva le affection del hepate (Kirtley). Es cognoscite cinque reportos autoptic (Harris). Le reporto autoptic de Field monstrava extense myocarditis interstitial focal, simile a illo vidite in sensibilitate a sulfonamido. Le morte reportate per Root esseva characterisate per un hepatitis que le pathologos distingueva ab le formas virusal e que esseva designate como toxic. Le caso de Field habeva etiam focal lesiones granulomatose del hepate, del splen, e del nodulos lymphatic.

In omne casos de lesiones hepatic, incluse un numero de casos resultante in le recovramento del patiente, le tests del function hepatic esseva normal excepte le test de van den Bergh in le presentia de jalnessa e le test del phosphatase alcalin del sero (Root). Le secunde de iste duo tests es apparentemente importantissime. Illo deberea esser usate plus precocemente in le observation consecutori de patientes tractate. Le possibilitate que eosinophilia annuncia le imminentia de un reaction toxic esseva mentionate per Field. In le discussion oral, Dr. Williams citava su experientia que thiouracil rende superflue le numeration routinari del cellulas sanguinee in le effortio de evitar leucopenia.

Iste prime reportos american de toxicitate rende obvie le facto que on debe expectar que le effectos de iste drogas va includer non solmente minor reactiones allergic, como per exemplo dermatitis, sed etiam sever alterationes generalisate. Apparentemente 1 g per die es generalmente considerate como un salve dos e de mantenentia, sed mesmo isto non es un garantia contra sequelas allergic, proque tal sequelas non es relationate al nivello del droga in le sanguine.

Iste symposio, in comparation con illo de martio 1956, reflecteva un considerabile augmento del cognoscentias relative al sulfonylureas, specialmente in le area del delimitation e delineation del mechanismos possibile de lor action.

Areas que require investigationes additional es le sequente: (a) Le differentias del comportamento de pyruvato sanguinee quando un sulfonylurea e quando insulina es administrate. (b) Le plus grande sensibilitate de animales adrenalectomisate in comparation con animales hypophysectomisate quando illos es exponite a iste drogas. (c) Le effecto del drogas in animales dispancreatisate e tractate con insulina. (d) Le question si le production de insulina o le livration de insulina es stimulate. (e) Le effecto de glucagon in le presentia de sulfonylureas super le production de glucosa per le hepate. (f) Le differentia in le effecto hypoglycemic post administration oral e intravenose. (g) Additional studios statistic in re le toxicitate del drogas.

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EDITORIALS

MYOCARDIAL METABOLISM IN DIABETES

Early studies on cardiac metabolism in diabetes have dealt with investigations on the isolated heart or the heart-lung preparation. Later the studies were extended to intermediary metabolism using tissue slices or homogenates. Already during the early phase of these investigations it was discovered that the diabetic heart in vitro had not lost its ability to use sugar. Using the hearts of depancreatized dogs in situ, a positive myocardial glucose balance was found. Later, tissue slices from hearts of diabetic dogs were found to utilize pyruvate and lactate less readily than slices from normal animals. Similarly, diminished utilization of C^{14}-labeled pyruvate was observed in both cardiac and diaphragmatic muscle of diabetic animals and the fraction of pyruvate converted to carbon dioxide was decreased. The addition of insulin had no influence on the conversion of labeled pyruvate to $C^{14}Co_2$ in cardiac muscle in vitro. The metabolic deficiency was found to extend also to utilization of fatty acids. This appeared to be related to the disturbance in carbohydrate metabolism, since the transfer of energy required for fatty acid synthesis seemed to be derived mainly from coupled reactions involving the simultaneous oxidation of some carbohydrate intermediate. Thus, in general, the diabetic state appeared to be accompanied by a marked decrease in the ability to synthesize fatty acids from glucose, lactate or pyruvate.

In experiments on the whole animal, deficient protein synthesis in diabetes was also found, since high fasting plasma amino acid levels were frequently present in patients with severe untreated diabetes. It appeared, therefore, that utilization of carbohydrates by the diabetic organism is diminished and that the diabetic organism is also deficient in utilization of fat and protein. Whether this is the result of disturbances in glucose transfer across membranes; or of interferences with the hexokinase reaction; or of oxidative phosphorylation; or of oxidative reactions within the Krebs cycle remains undetermined.

Coronary sinus catherization has made it possible to investigate over-all metabolic changes of the diabetic heart in patients with diabetes mellitus and in dogs made diabetic with alloxan. This type of investigation bears a close similarity to earlier methods of study, such as the isolated heart or the heart-lung preparation. This technic has its limitations. For example, one can only determine the balance of foodstuffs across the heart, and conclusions on the fate of these substrates in the cell, the participation of enzyme systems or the localization of metabolic defects, are not possible. However, these balance studies are carried out on the heart beating in its natural environment.

The results obtained on the diabetic heart of humans and dogs with this technic have shown that myocardial usage of carbohydrates is reduced and that utilization of noncarbohydrate material is increased. Apparently the heart in situ is not exempt from the most important metabolic defect in diabetes, that of deficient utilization of carbohydrate. The decreased ability of the diabetic human and dog's heart to utilize lactate is particularly conspicuous. The marked reduction of myocardial usage of lactate is primarily responsible for the reduction in the total amount of energy available from the carbohydrate fraction. This agrees with data obtained on the intact diabetic animal. For instance, the arterial lactate concentration of depancreatized dogs reaches higher values during exercise than in the nondiabetic animal. Pyruvate extraction by the diabetic human and dog's heart is within normal limits. However, when one considers that the normal pyruvate extraction occurs in the presence of a significant elevation in arterial concentration of pyruvate, this finding suggests diminished usage of pyruvate. It had been previously shown that tissue slices from diabetic dogs utilize pyruvate and lactate less readily than those from normal animals. Furthermore, the rate of oxidation of acetate and pyruvate to carbon dioxide was found to be diminished in diaphragms from alloxan diabetic rats.

In the face of reduced carbohydrate utilization, a relatively large amount of noncarbohydrate material must be used by the heart for energy production. This is actually the case since the human heart in patients with diabetes mellitus extracts a significantly greater amount of fatty acids than does the nondiabetic heart. Fatty acids may be stored by the diabetic heart since the oxygen extraction ratio of fatty acids which represents their contribution to the total myocardial oxygen extraction, is above 100 per cent. The usage of ketone bodies by the diabetic heart is also increased.

If, under those conditions, insulin is to correct the metabolic defects found in the diabetic heart, it should result in a relative increase in myocardial utilization of fatty acids and ketones. The hormone results in a significant fall in blood sugar, but this occurs without changes in myocardial usage and extraction of glucose. This implies that insulin causes a relative increase in myocardial glucose utilization. After insulin injections into diabetic dogs, the concentration of pyruvate in coronary vein blood frequently exceeds that in arterial blood. This diminished myocardial uptake of pyruvate by the heart muscle may be an indication of increased catabolism of endogenous carbohydrate in heart muscle, initiated by a rapidly falling blood sugar level in these severely diabetic organisms. This may be due to a block between pyruvate and the Krebs cycle resulting from a reduced thiamine content of heart muscle. However, the condensation of pyruvic acid with oxalacetic acid is so complex that it is impossible to speculate on the exact location of this defect. Equally surprising was the finding that insulin failed to correct the metabolic defect responsible for diminished myocardial lactate usage. Arterial lactate concentration rose, but myocardial extraction of lactate hardly changed. Insulin resulted in a fall in the blood concentration in fatty acids without significantly affecting their myocardial usage or extraction. It appeared likely that the fall in blood concentration of fatty acids was due to a decreased mobilization from fat deposits.

The results obtained on the diabetic heart in situ demonstrate a great variety of metabolic defects. The diabetic heart is deficient in glucose, pyruvate and lactate utilization. In addition, the defect extends to the metabolism of protein and fat. It is unlikely, however, that these diffuse changes in energy production result in disturbances in energy utilization of the heart. The fundamental importance of diabetes in cardiology does not rest on primary metabolic disease of heart muscle but rather on the frequency with which diabetes results in coronary vascular changes and its complications.

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SCIENTIFIC PROGRAMS OF AFFILIATE ASSOCIATIONS

The editors of DIABETES desire to call the attention of members of the Affiliate Associations of the American Diabetes Association to the desirability of using the *Journal* as a vehicle for announcing scientific programs of the Affiliates.

Recent experience of the New York Diabetes Association in which more than 900 persons from twenty-two states attended the Fourth Annual Symposium of this

Affiliate Association on Oct. 12, 1956, was partly due to the announcement of its program through the pages of this *Journal* and announcements sent to the membership of the American Diabetes Association.

As the subscription list of DIABETES grows, it is reasonable to expect the readers to consult its pages for news of interest concerning scientific presentations dealing with diabetes in various parts of the country. Since the *Journal* is bimonthly, announcements of such programs must be sent not later than three or four months in advance of the expected program date.

REPORT OF THE COMMITTEE ON CAMPS

In 1925, within three years after the initial clinical use of insulin, the first camp for diabetic children was founded by the late Dr. Leonard F. C. Wendt in Michigan. Insulin gave the diabetic child not only the precious gift of prolonged life, but the physical stamina, nutrition and freedom from restricted routine which allowed him to participate in camping activities.

Following Dr. Wendt's pioneering spirit, a number of other equally courageous physicians opened camps for diabetics: John of Cleveland, Joslin and White of Boston, and Fischer and Green of New York City. By 1931 there were five well established camps for diabetic children throughout the country.

Twenty-six years later this number has grown to twenty-six. The capacity and scope of activities of the camps have increased each year. The Clara Barton Birthplace Camp, as an example, took twenty-eight girls in 1930. Today it can accommodate groups of over seventy girls for five periods of two weeks each. Camps are now located in every section of the country and it is safe to say that no diabetic child in the United States is more than one day's trip by auto, bus or train from a camp. The metropolitan areas of Boston, New York City, Philadelphia, Cleveland, Toronto, Chicago, Detroit, Indianapolis, St. Louis, Milwaukee, Dallas, Los Angeles, San Francisco, and Seattle have diabetic camps within commuting distance.

The American Diabetes Association has always encouraged the establishment of camps for diabetic children. At no time, however, has the Association endeavored to operate or foster any single camp. The Committee on Camps of the Association has been composed of members interested in furthering the cause of camping. This committee has reviewed and discussed pertinent problems. The meetings serve as a clearing house through which knowledge, experience and solutions of common problems are exchanged and passed along to the many camps. A Subcommittee on Medical Standards was appointed in 1955. Its report entitled "Suggested Medical Standards For Camps For Diabetic Children" presents a significant milestone in the guidance of camping for diabetics. The report is the result of an integrated study of a compendium of information from all established camps. As indicated, the "standards" are not meant to be binding in any way. Neither attempt at comparison, rating, approval, or disapproval has been made.

It will be apparent to anyone acquainted with diabetic camps that most camps have some of the ideal features intermixed with some of the minimal standards. Most camps have some features which surpass even the "ideal." Few, if any, camps provide only the minimal requirements. It can be hoped that the time will come when the advancement of diabetic camping reaches such a level that these ideal standards will be minimal standards and a new even higher set of "ideals" can be sought. This report can serve as a guide and stimulus to both established and embryonic camps. A number of the points made in the report are deserving of special comment.

Ideal entrance requirements are little more than ordinary good medical care of the diabetic child would dictate. The implementation of these requirements could serve as a means of educating all physicians in the proper routine care of diabetic children. It could also serve to emphasize the importance of periodic examinations, immunization and dietary evaluation. The great majority of our school systems require periodic medical examination and routine immunizations against diphtheria, pertussis and smallpox. It is thus well within the prerogative of a camp for diabetic children to require these, and, in addition, the other suggested procedures such as Salk vaccination, chest X rays and emotional evaluation. A careful screening program plus a preventative medical program could save an entire camp from a catastrophic epidemic.

The suggestion that a comment be made upon the emotional status of the child is of utmost importance. Emotional problems are common in the diabetic child. If the camp personnel can be alerted to specific problems of individual children they can prepare and, many times, save the individual or his whole cabin group from an unpleasant camp experience. In many instances the medical social worker of the camper's hospital can supply valuable psychological or behavioral information. A maladjusted camper can spoil the summer for him-

self and all of those around him.

Sponsorship of diabetic camps differs from camp to camp. Many camps are sponsored by Affiliates of the American Diabetes Association. Others are sponsored by local civic groups or individuals with civic or church support. The suggested "table of organization" is applicable and useful for any of these individuals or groups. Proper integration of physicians and lay people is of paramount importance. A physician cannot be a camp director nor recreation director. By the same token an experienced camp director cannot provide the medical supervision and planning required in a diabetic camp. It is, however, important that both the medical and lay personnel of a camp be well oriented as to each other's problems. This orientation is dynamic and can be achieved only through frequent meetings of all individuals participating in the program. Meetings prior to, during, and following the camp season are essential. In this way potential problems may be aborted, imminent problems solved and mistakes corrected for future operations. Ultimate authority as suggested should be vested in the medical director. He is medically responsible for the welfare of the children, and the unpleasant spectre of malpractice must always be kept in mind when operating this type of camp.

There is little question that every camp should have at least one resident physician. The advantages to the operation of the camp are obvious. Recognition of the advantages to the resident are not as widely recognized. There is no place other than at a summer camp for diabetic children that a young or (older) physician can have the opportunity of observing and caring for from 15 to 100 juvenile diabetics. Certainly, no hospital has more than 10 to 15 juvenile diabetics at any one time. In addition, each of these children is participating in a full, normal activity program which is impossible in the hospital. The resident is thus given the opportunity to observe the day to day problems of control in a group of active diabetic children. With one or more residents in attendance at a camp it is then incumbent upon the physician members of the camp committee to visit camp frequently to conduct "teaching rounds" for the residents. The privilege and responsibility of the physicians is then to impart their knowledge of diabetes to the resident staff. If this type of program is worked out in the camps, the universities and teaching centers may ultimately include a period in a diabetic camp as part of their residency program in internal medicine and pediatrics.

Dietary management is the keystone of any camp, diabetic or otherwise. Preparation and variety of foods are critically examined by all campers. The diabetic diet routine for a large camp, almost of necessity, requires the services of a well qualified dietitian. Clever meal planning is important in rounding out a pleasant and happy camp experience for the campers. If there is imagination in planning a great variety of food, the children soon forget that they are on a mandatory diet. A smaller camp's diet program could be run by remote control. Menus for the whole camp season could be set up by a trained dietitian prior to camp. A well trained staff of cooks and servers could then prepare and portion out the food. Again a dietitian is important for economic purchasing of foods and for keeping a cost accounting system in operation.

Counselor selection is of utmost importance and is of course the responsibility of the lay director. Once a good staff of counselors is obtained, it is the responsibility of the medical director to orient and train the counselors in the problems encountered in the care of a diabetic child. They must be briefed on: 1. Hypoglycemia, its causes, symptoms and immediate treatment. 2. Behavior problems commonly encountered. 3. Principles of diabetic control. 4. How to answer specific questions from the children and to whom to refer them for further answers. These and other points necessary for elementary understanding of diabetes in children should be taught to the counselors prior to the opening of camp.

In general, camping for diabetic children and adolescents is expanding throughout the United States. This is a logical and commendable trend. It must, however, be kept in mind that mere expansion in numbers is neither wise nor desirable. The existing facilities should be utilized to their reasonable capacity. Any group or individual contemplating a camp would be wise to study existing facilities within the area and make some estimate of the number of potential campers residing in the area. It may be possible in many cases to combine the efforts of two or more groups in one camp. A combination of this type would result in better facilities, personnel, and greater capacity.

By making continual surveys of diabetic camps, their growth and problems, it is hoped that the Committee on Camps will be able to continue to serve the diabetic population.

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John P. Peters
1887-1955

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John Punnett Peters died on Dec. 29, 1955, at the age of sixty-eight, bringing to a close an illustrious career in scientific and academic medicine. Dr. Peters was one of those leaders in American medicine who exemplified in his own life and work the ideal qualities of the scientist, teacher, physician, and citizen.

Were Thomas Carlyle alive today to add to his lecture series "On Heroes, Hero-Worship, and the Heroic in History" he could well use him as "The Hero as Scientist." Beginning in 1916 and continuing for thirty-nine years, a steady stream of articles, monographs, and books, 199 in all, emanated from his pen, each one a significant contribution to the science of medicine. No other single man has done more to make physiology and biochemistry an integral part of medicine today. For thirty-four years at Yale, first as Associate Professor and then as John Slade Ely Professor of Internal Medicine, he maintained a center dedicated to the application of physiological and biochemical knowledge to the diagnosis and treatment of disease in man. In his later years he often quoted Pope's line: "The proper study of mankind is man" to emphasize the importance of directing medical research toward man himself, rather than toward other species. Not one of his own investigations was made on animals. His method of experimental approach remained essentially unchanged throughout his career and could well serve as a model to all clinical investigators. Of prime importance to him was an intimate and firsthand knowledge of the patient and his disease. In the laboratory he insisted on accurate technics, making Yale a model of what a good laboratory of quantitative clinical chemistry should be. He had an intense scorn for those hospitals, no matter how famous, where double standards for "routine" and "research" chemical technics existed, for in his own laboratory the same procedures were applied to both, and duplicate determinations were done without exception in all analyses. This enabled him at any time to use data obtained from patients at New Haven Hospital for the study and interpretation of disease processes. Accurate and detailed records were kept on every determination, often in his own hand, so that he could describe quantitatively the various biochemical changes that take place in each group of diseases. Description of data was only the beginning of his scientific contributions. His keen critical evaluation of results of others as well as of his own, and his ability to synthesize and clarify his own points of view resulted in major advances in concept and therapy of metabolic disorders. Finally, in attempting to describe his scientific approach, it is worthy of note that he seldom resorted to planned experiments in humans which would involve any hazard, great or insignificant. He was content with studying the changes that nature herself performed in the guise of disease. His research covered a wide spectrum of subjects, including acid-base equilibrium, respiratory physiology, water and electrolyte metabolism, renal disease, thyroid function, pregnancy, cardiac failure, gastrointestinal metabolic disorders, diabetes mellitus, and other endocrine disturbances, and the metabolism of protein, fats and carbohydrates. Dr. Peters' scientific contributions played an integral part in the development and growth of quantitative clinical chemistry, in the broadest sense of the term, from its smallest beginnings to the stature and maturity it fulfills in medicine today. No other single work epitomizes this so well as the monumental "Quantitative Clinical Chemistry" written in 1931 and 1932 with Donald Van Slyke and published in two volumes, "Interpretations" and "Methods." Although he had time to revise only part of this by 1946, it still remains the classic in its field.

John Peters' contributions to diabetes encompassed almost every facet of the problem, twenty-six papers relating to the subject testifying to his interest. An experienced clinician, alert to the nuances of the disease from patient to patient, he strengthened his knowledge by continuous observations in the office and at the bedside over a twenty-four-year period. He had a firsthand experience with the natural history of diabetes and as such was fully aware of the problems of clinical management and the development of the dreaded vascular complications. He coupled this with his laboratory investigations in various spheres. In 1917 he wrote about the relation of adrenalin hyperglycemia to decreased alkali reserve of the blood. In 1923 the effect of infection in diabetes was reviewed. He was interested in the

plasma proteins, particularly in diabetic acidosis, finding that the actual levels were often obscured by the hemoconcentration present. These studies clarified the role of hypoproteinemia and excessive alkali administration in the causation of "diabetic edema." The nutritional disturbances seen in severe uncontrolled diabetes focused his attention on the effects of starvation, culminating in his stimulating paper in 1945 on "Starvation Diabetes, the Reason for the Use of Glucose in the Treatment of Diabetic Acidosis" (*Yale J. Biol. & Med. 17*:705, 1945). This masterful summary of a controversial aspect of treatment is concluded with the following statement: "The ultimate solution of this question must rest upon the sound assemblage of physiological evidence, supported by controlled clinical observations, not by *ad hoc* arguments from particular cases. An attempt has been made to array in some semblance of order the most significant items in the great mass of evidence that carbohydrate should be used in the treatment of diabetic acidosis because it promotes the oxidation of glycogen, supplements the action of insulin, reduces the destruction of protein, and diminishes the production of ketone bodies." The work of Seldin and Tarail in his laboratory caused him to consider the undesirable features of glucose administration—polyuria, loss of salt, and dehydration. He avoided this dilemma in 1954 (*Yale J. Biol. & Med. 27*:152, 1954) by advocating the substitution of fructose for glucose, based on physiological and clinical evidence that "fructose is removed from the blood of the patient with diabetic acidosis as rapidly as it is from the blood of a normal person. . . . Moreover, perhaps because of the advanced point at which it enters the chain of oxidative reactions, a certain proportion of it can be utilized. Although it may contribute to blood glucose, if given rapidly, moderate amounts produce little or no hyperglycemia. Fructose solution is, therefore, an ideal vehicle for the parenteral administration of water in diabetic acidosis and at the same time provides some utilizable sugar."

Other aspects of diabetic acidosis were investigated. As early as 1925, in a study of fifty-three cases of diabetic acidosis, he proved that "in profound diabetic toxemia the salt content of the blood and probably that of the tissues is seriously depleted' 'thus laying the foundation for the routine use of saline in treatment. The importance of these observations was overlooked by some of the leading diabetic clinics for many years. In 1946 a careful analysis of the 188 cases of diabetic acidosis observed over a period of more than twenty years revealed a striking correlation between the degree of salt depletion, the occurrence of peripheral vascular collapse, and the mortality rate. Here again careful clinical and laboratory observations resulted in important contributions to rational therapy, the use of saline and plasma expanders (*Yale J. Biol. & Med. 18*:405, 1946).

He next became interested in some of the electrolyte changes in red blood cells in diabetic acidosis, finding that there was extreme depletion of phosphates and base (*Am. J. Phys. 149*:667, 1947). The impairment of the glycolytic process in the blood in some cases of severe diabetic acidosis was found to be correlated with depletion of organic acid-soluble phosphate (*J. Applied Physiol. 5*:647, 1953).

Beginning in 1934 the lipid fractions of the serum in diabetic acidosis and in controlled diabetes were investigated (*J. Clin. Invest. 13*:237, 1934; *14*:579, 1935; *Metabolism 2*:120, 1953). The elevation of lipids found in diabetic acidosis was largely due to hemoconcentration. Detailed analysis of seventy-nine diabetic patients not in acidosis showed that the level of cholesterol was not related to severity of diabetes, the amount of fat in diet or the degree of arteriosclerosis. Here again the laboratory helped the clinician clarify his problems, this time in the area of fat metabolism.

Significant contributions were made in other areas related to diabetes. Arteriovenous blood sugar differences in normal and diabetic subjects and the effect of insulin were determined (*J. Biol. Chem. 80*:269, 1928; *Arch. Int. Med. 43*:633, 1929). Four cases of intercapillary glomerulosclerosis with autopsy findings were reported in 1939 (*Arch. Int. Med. 64*:1252, 1939). The rapid intravenous tolerance test, employing 50 cc. of 50 per cent glucose, was described in 1941 (*J. Clin. Invest. 20*:507, 1941). This test is now a standard diagnostic procedure in many clinics, because of the avoidance of variations in intestinal absorption of glucose, and its reproducibility under standard conditions. The problem of diabetes and pregnancy was not overlooked, the clinical experience being reviewed and systematically analyzed (*Yale J. Biol. & Med. 16*:151, 1943).

Last, but by no means least, must be cited the articles summarizing his ideas on the management of diabetes (*Rhode Island Med. 21*:1, 1938; *New Internat. Clinics 2*:171, 1941; *Yale J. Biol. & Med. 27*:53, 1954). The 1954 article can justifiably be called a "classic," written with elegance and felicitous phrase and emphasizing rational therapy based on sound physiological and biochemical facts. This is combined with the realization that the doctor must retain the humanistic and personal point of view. A few quotations will illustrate his approach: regarding objectives, "The objective in the treatment of any chronic disease should be

to enable the patient to enjoy as full and untrammeled a life as possible. Medicine should not be entirely negative and restrictive. It should especially avoid emptying life of the features that give enjoyment and a sense of accomplishment. The therapeutic regime should not make the patient conspicuous; his ailments are his own business. With insulin preparations that are now available the diabetic should be enabled to pursue a normal course of life with no lets or hindrances other than adherence to a diet, injection of insulin, and analysis of urine, except as these may be necessary to meet complicating or associated disorders. The patient with advanced arterial disease cannot profitably return to digging ditches. Since all the activities and vicissitudes of life influence the course of diabetes, no satisfactory therapeutic regime can be established or adjusted in a hospital. Hospitalization is necessary or desirable only for the management of complicating conditions or emergencies." On diet: "To place the full onus for infractions of dietary and other regulations upon the patient is an evasion of responsibility by the physician, whose prior assumption should be that the regime has been improperly devised. To upbraid the patient as if weaknesses were sins may destroy the frank relations that should prevail between physician and patient. To invoke fear is cruel. Lapses should be met with sympathy and understanding, their consequences explained with kindly reason. Self-righteousness on the part of the physician does not benefit the patient. An honest compromise, even though not altogether desirable, may be necessary. A little liberty is better than license." On complications: "A careful investigation of the natural history of diabetes promises greater returns. The sad realization has been reached that a large proportion of children afflicted with the disorder follow the pattern of adults with respect to vascular disease. This majority in both groups is, however, no more important than the minority that mysteriously escapes these evils, or, since the life history of the young diabetic is still to be written, has managed to survive without them far beyond the general expectation. It is too early to congratulate ourselves and somewhat ridiculous to blame patients for the incidence of these associated complicating conditions. Some of the most medically virtuous have succumbed early, while rascals have escaped. I will not say that only the good die young; but experience has convinced me that in this, as in many another panel of life, virtue too often has to be its own and only reward."

No assessment of Dr. Peters' contribution to diabetes can close without citing his critical and encyclopedic review of carbohydrate metabolism in *Quantitative Clinical Chemistry.* In the first edition published in 1931 this covered 147 pages with 481 references. The rapid growth of knowledge in the field is shown by the increase by 1946 to 273 pages with 1,316 references. Every sentence in the review bears witness to his ability to analyze and synthesize this vast area of metabolism.

Jack Peters' place in American medicine is further strengthened by his influence on students, both undergraduate and postgraduate. In teaching he always aimed at the highest level because he firmly believed that medical students should be treated as adults who were not to be spoon-fed but rather stimulated and provoked to think. Many a student still recalls vividly his quick and caustic remarks when opinions, unsupported by logic or data, were voiced. This uncompromising attitude toward ignorance or faulty reasoning was directed not only at students, but at all levels, so that even his colleagues and peers chose their words with care in his presence. His famous "Metabolism Rounds" at Yale, held thrice weekly, were memorable for many reasons. He spoke softly and with little facial expression, so that from a distance his rounds could be identified by the huddle of his disciples straining to catch his every word (lip-reading became an invaluable aid!). His nimble and facile mind, fortified by his tremendous knowledge of experimental clinical medicine, would frequently leap-frog many steps in the reasoning that culminated in the often brilliant interpretations or diagnoses at the bedside, to the despair of those less well-versed in the field. But as one's own knowledge increased, the appreciation of his abilities increased geometrically, and it is perhaps the young men who were trained in his metabolic service and laboratory who owe him the greatest debt for the stimulation and insight he provided into clinical medicine and investigation. The later years of his life were blessed by the knowledge that so many of his "boys" had gone out to other medical centers to establish islands of teaching and investigation in the metabolic field.

Those who did not see him function in his own hospital environment did not always appreciate his abilities as a physician. In a famous radio debate on the social aspects of medicine late in the thirties, the defender of the status quo of the practice of medicine, confronted by the overwhelming logic and carefully considered facts presented by Dr. Peters, retorted by saying that Dr. Peters might be an authority in chemistry but was not qualified to give any opinion on the subject under debate because he was a "professor" who knew little about patients and disease. In reality Dr.

Peters was an able and expert physician, skilled in every aspect of the care of the patient not only on the organic side but also in the psychological sphere. He was always quick to point out the importance of the emotional aspects, especially the impact on metabolic functions, as in diabetes and thyroid disease. His house officers soon learned not to relegate every emotional problem to the psychiatrist, for Dr. Peters believed strongly that the physician responsible for the organic aspects of disease was best qualified to evaluate these factors. As a full-time teacher on salary, he never collected any fees for himself, yet every patient on his service or whom he saw in his office received from him the best possible type of medical care. This individual approach to the patient, epitomizing the highest level of physician relationship, nevertheless was coupled in his mind with the recognition that the inevitable increase in laboratory medicine would force a great expansion in institutional equipment and in group cooperation of professional and other ancillary medical personnel. He felt strongly that the day had passed when the problems of medicine could be handled by the individual private physician practicing entirely out of the little black bag. Aware also of the economic consequences of the enormous growth in medical knowledge he entered actively into the controversies surrounding methods of medical care and from 1927 until 1954 served as secretary of the Committee of Physicians for the Improvement of Medical Care. As such he frequently became the target for vilification and slander by the more conservative members of organized medicine. He was convinced that comprehensive health coverage was not only necessary but inevitable and that it was the responsibility of physicians to experiment in that direction, just as much as in the problem of disease itself. His great fear was that the medical profession, by blind opposition to change, would lose its opportunity to control and improve the practice of medicine, thus allowing those lay people concerned only with the economics of medicine rather than quality of medical care to develop the pattern and rules.

It was as a citizen that Dr. Peters, the "Hero as Scientist," was best known to the public in the last few years. During World War II he conducted investigations for the Air Force on survival rations and for the National Research Council on nutrition in injury and disease. The Quartermaster Corps used him as a Consultant and after the War he was appointed a Consultant to the Army Medical School. From 1947 to 1954 he served on the Study Section on Endocrinology and Metabolism of the National Institutes of Health in the Public Health Service. His advice was sought on all sides by many interested in metabolic research and he never failed to give unstintingly of his time and knowledge.

Socially and politically he was a liberal and progressive, a natural consequence of his distinguished American heritage which dated back more than three hundred years to prerevolutionary days. There is a letter on record of one Abigail Peters written to the presiding judge of the Salem assize, protesting the witchcraft trials. His father was an eminent member of the clergy and an archeologist of note who was very active in civic affairs and who had a reputation for being a militant advocate of causes he considered just, often upsetting the complacency of his wealthy New York parish. His scholarship, broad interests, and deep moral sense of social responsibility had a significant influence on his son. With the upsurge of McCarthyism after World War II, Dr. Peters' liberalism became suspect and on the basis of anonymous information he was discharged in 1953 from his position as consultant to the U. S. Public Health Service on its study section for metabolism. This was despite the fact that he had received loyalty clearance in 1949 and in 1952. His case, supported by professors from the Yale Law School and a former U.S. Attorney General, was carried through to the Supreme Court, and resulted in complete personal vindication. To his great sorrow, however, the court failed to rule decisively on the fundamental constitutional question of the right of an accused person to face and crossexamine his accusers. Nevertheless, he had succeeded in calling attention forcibly to important questions of possible encroachment on the basic civil liberties of his fellow citizens.

To describe Dr. Peters as a person is difficult. Dr. J. Russell Elkinton's remarks to the Interurban Club must be quoted: "John Peters was not a simple man in his mental or emotional life, nor was he always an easy man with whom to be associated. Almost every activity that he undertook he undertook with intensity. He was intense, nay even passionate, whether he was playing tennis, growing roses, playing the piano, or indulging in controversy with one of his cherished scientific or economic adversaries. His capacity for criticism was tremendous and often devastating. Perhaps one of the sad ironies of his life was that his criticism was often so penetrating that it was feared, and hence his advice was less sought after and his wisdom was less influential in the world of American medicine than it might otherwise have been. But underneath his critical exterior he had a kind heart; sooner or later

each of his associates came to appreciate this fact as "The Boss" helped him to meet some of the vicissitudes of life.

The genius of John P. Peters consisted to a great extent in the coupling of a razor-sharp critical mind with a profound knowledge of the experimental literature; it can be said that rarely did a problem probed by him remain entirely unclarified, and seldom did the probing thereof fail to open new vistas of inquiry. And yet he was a more complex individual than that. The essence of the man lay in his integrity. Above all else he abhorred hypocrisy, he abominated sham, he deplored complacency. And if he appeared to be hypercritical and to be in constant revolt against the established order, the cause usually lay in his wholehearted pursuit of the truth as he saw it. Thus to the profession of medicine and to society he brought a mind and a fierce purpose, and his contributions were commensurate."

And finally, the remarks of his attorney, Fowler Harper, Professor of Law at Yale, at the Memorial Service in New Haven serve further to illuminate the portrait of the man: "I think I saw with some clarity the many contradictions of his character—the kindliness and the steel, the simplicity and the urbanity, the intricate and the complicated personality, the precision-like mechanism of his mind, the softness and the warmth, the flint-like quality of his courage. In man's long struggle to civilize himself, it would be hard to find better evidence of success."

CORRESPONDENCE

To the Editor:

As I have stated elsewhere, observations of symptomatic differences made it evident from the outset that the hypoglycemia of the sulfonylurea drugs is in no sense an insulin hypoglycemia. The mere fact that they are not powerful enough to prevail against the profound collapse of carbohydrate metabolism resulting from total absence of insulin does not imply that this action is insulin-mediated. Insulin accelerates tissue uptake of sugar at the cost of depletion of liver glycogen, while plausible evidence seems to point to an opposite effect of these drugs, namely an action on the liver causing hypoglycemia by increased glycogen storage and diminished discharge of glucose, with less evident augmentation of peripheral uptake. This or any other artificial mechanism may be ground for suspicion of a toxic interference with metabolism and the likelihood of overt injury in the course of time. While it may be granted that the hypoglycemic action is as unphysiologic as the antibacterial action of sulfonamides, the fear of toxicity except in a few sensitive individuals may be countered by the following considerations. First, the usual effect of hepatotoxins is increase of fat and reduction of glycogen in the liver, and no agent initially increasing glycogen is known to lead to necrosis or cirrhosis. Second, laboratory studies may overlook the fact that utilization of sugar is actually brought about; that is, sugar previously lost in the urine is metabolized, presumably in the peripheral tissues. Third, with recollections of the early debate of overproduction versus underconsumption of sugar, and the modern view that both processes are involved in diabetes, it is conceivable that a slower and more orderly flow of sugar to the blood may precisely suit the need of some patients possessing a moderate supply of autogenous insulin. These theoretical considerations reinforce a clinical experience that small doses of these drugs in suitable patients are truly beneficial.

FREDERICK M. ALLEN, M.D.
New York, New York

BOOK REVIEWS

COWDRY'S PROBLEMS OF AGEING-BIOLOGICAL AND MEDICAL ASPECTS. *Edited by A. I. Lansing, Ph.D., Associate Professor of Anatomy, Washington University School of Medicine, St. Louis, Mo. $15.00, pp. 1061, 3rd Edition, The Williams and Wilkins Co., Baltimore, 1952.*

This book, first published in 1939, has been brought up to date. It is timely, well conceived, and splendidly executed. Every conceivable aspect of the problems of ageing has been ably presented. The forty-eight contributors have been well selected. The magnitude of

the problems is appalling. The several essays of the book point up this fact. They present the current knowledge of the various aspects and indicate lines of research for solving the problems. The contributors do not pretend to have all the answers. As one reads the essays he realizes the vastness of knowledge to be acquired, and he is humbled by the relatively small inroads we have made to discover why people grow old. There would be no point of discussing the individual essays. The average clinician might gain something of practical value in the last few essays, but the great value of the book is for those working in the field of geriatrics, particularly the research field, as background material. It must be admitted, however, that even these men and women, forging ahead in limited fields, must delve even deeper into the work of past and present investigators in the pursuance of their research activities.

The format and typography are excellent, and the illustrations are above reproach.

DIAGNOSIS AND TREATMENT OF VASCULAR DISORDERS. *Edited by Saul S. Samuels, M.D., $16.00, pp. 621, The Williams and Wilkins Co., Baltimore, 1956.*

In recent years more and more attention has been focused upon problems of circulation. A great deal has been learned about the blood vessels and, as the editor states, the study and treatment of diseases of the blood vessels has come into its own as a specialty, and he suggests the term "Angiology" for this specialty containing within it medical and surgical components.

In this book, seventeen experts have contributed one or more chapters and the subject has been approached in a logical fashion. The first four chapters deal with the anatomy and physiology of the peripheral blood vessels and examination of the patient. There is then an excellent chapter on angiography. The various vascular disorders are reviewed in the succeeding chapters and the book ends with a brief discussion of medicolegal aspects. It is a well-published text with many excellent illustrations and is written in a lucid style adapted for easy reading.

In any book of mixed authorship, the pace is apt to be uneven and in this volume at times there seems to be a lack of correlation of the opinions of the various authors, as is exemplified in relation to the indications for sympathectomy in the treatment of intermittent claudication. Certain diagnostic and therapeutic technics appear to be overemphasized while others are given a rather cursory review.

The terminology used in certain chapters is apt to be confusing to some readers as it differs from the more usual concepts. For example, the term "Juvenile Obliterative Arteritis" is used in one of the chapters as synonymous with "Thromboangiitis Obliterans" whereas later in the book there is an entire chapter on "Thromboangiitis Obliterans." "Endarteritis Obliterans" is the subject of one chapter and has been considered an entity by the author, but there is no uniform agreement among authorities in the field that such an entity exists. In relation to the question of terminology, the book emphasizes the need of more uniform classification of vascular disease on an international basis.

Most vascular problems are discussed but a few, such as "Temporal Arteritis," have not been considered.

In the opinion of the reviewer a book of this type is of value inasmuch as it presents to the reader the opinions of a number of authorities in this field. It is both a stimulating and provocative volume because it serves to point out the many unsettled problems in the field of angiology.

HUMAN PHYSIOLOGY. *Edited by Bernardo A. Houssay, M.D., Juan T. Lewis, M.D., Oscar Orias, M.D., Eduardo Braun-Menendez, M.D., Enrique Hug, M.D., Virgilio G. Foglia, M.D., and Luis F. Leloir, M.D., $12.00, pp. 1177, 2nd edition, McGraw-Hill Book Co., Inc., New York, 1955.*

As in the first edition of the textbook, *Human Physiology,* the authors have succeeded in presenting the current concepts in physiology in a concise and lucid manner. The material has been brought up to date while the basic organization of the first edition has been retained. The authors, who are all teachers and active investigators, have presented a balanced treatment of the various areas of physiology and have not placed undue emphasis on their own particular fields of interest.

The text is divided into the following sections: "The Internal Environment and the Blood"; "The Circulation"; "Respiration"; "Digestion"; "Metabolism and Nutrition"; "Internal Secretions"; "Reproduction"; "The Formation and Excretion of Urine"; and "The Nervous System." Each section and chapter presents a logical development of the evidence in support of currently accepted views. Where the actual experiments or data are not given in the text, adequate references are provided at the end of the individual chapter. Thus the inquiring student may examine the data upon which many of the fundamental concepts are based.

While most subjects are treated quite adequately, as would be expected the sections dealing with the internal secretions and with the regulation of carbohydrate metabolism are especially well presented. However, the chap-

ters dealing with carbohydrate and fat metabolism are rather concise and already out of date. This is not surprising in the light of recent progress made in these areas.

While primarily a textbook for medical students, the book will also be useful to the practitioner who wants to refresh his memory with regard to a particular aspect of physiology. In addition the references to many recent review articles will aid the reader in bringing his knowledge up to date.

The appearance of this second edition of *Human Physiology* should be welcomed by teachers concerned with the teaching of physiology to medical students as well as by practicing physicians.

CORONARY HEART DISEASE IN YOUNG ADULTS: *A Multidisciplinary Study. By Menard M. Gertler, M.D., Paul D. White, M.D., and Others; $5.00, pp. 218, published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1954.*

This is the report of an exhaustive study of a group of one hundred ambulatory patients with a history of myocardial infarction at least six months earlier, who at the time of infarction ranged in age from 22 years through 40 years. Ninety-seven were men and three women. There were 146 unmatched controls and a group of matched controls for the 97 male patients, consisting of 74 men from the unmatched control group and 23 additional men. The clinical findings were not impressive. The use of alcohol or tobacco could not be implicated as an etiologic factor. Heredity seemed to be important, but the mode of gene transference or inheritance and the degree of penetrance were not clarified. Diabetes was not mentioned. Apparently none of the patients had it. Of particular interest was the study of morphologic characteristics, endomorphic mesomorphs predominating. This part of the study was not complete. Athletic ratings were higher and the incidence of managerial occupations greater in the coronary group. The coronary patients were less masculine in their final scores than the matched control group. Endocrine studies limited to a thyroid and a testicular-adrenal survey based on twenty-four-hour urinary sterone excretion were inconclusive. Mean total cholesterol levels in the serum were higher in the coronary group, but a threshold level for coronary heart disease could not be said to exist. An important additional factor appeared to be the serum phospholipid and its relation as a colloidal stabilizer of the serum cholesterol. The phospholipids showed an increase with age in the normal group but did not keep pace with age in the coronary group. The serum uric acid was found to be higher in the coronary patients. The increased levels of cholesterol were highest in the mesomorphs, the increased uric acid in the endomorphs. As to diet, the controls ingested more cholesterol than the coronary patients. A study of the oxidation-reduction potentials of saliva showed a faster rate of change per minute in the coronary patients during the entire procedure.

Although on the whole there were suggestive differences between the coronary and control groups, no conclusions of a fundamental nature were possible. The book is well written and definitely worth reading.

PERSPECTIVES IN PHYSIOLOGY. *Edited by Ilza Veith, Assistant Professor of the History of Medicine, University of Chicago; $3.00, pp. 172, American Physiological Society, Washington, D. C., 1954.*

This small volume is a compilation of papers dealing with the science of physiology as it exists today and as it may exist in the future. Contributing to the monograph are leading physiologists of fourteen countries. Following an introduction by Adrian, there are five papers describing physiology and its relation to the other sciences. Bykov discusses the views on research in physiology in Russia. Best gives an account of how he feels a department of physiology should be organized.

The remaining twelve papers are each from one country and present the history and current status of physiology in that nation. Houssay speaks freely on the difficulties encountered in the development of physiology in Argentina. Hoffman elaborates on the hindrances arising during the Nazi regime in Germany. The general tenor reflects a universal lack of money, especially for personnel. However, enthusiasm for the future is voiced by all essayists. After reading this paper one cannot help but be impressed by the opulent environment that surrounds physiology in the United States.

Dr. Adrian states, "Whatever else it may do, I think this Symposium ought to make interesting reading fifty or even one hundred years hence." This little book makes interesting reading right now and is highly recommended to all interested in biologic research. It should be a part of all physiology libraries.

THE COMPLETE BOOK OF LOW CALORIE COOKING. *By Leonard Louis Levinson. $4.75, pp. 320, Hawthorn Books, Inc., New York, May 1956.*

The author's purpose, as expressed in the preface of his book, is to offer not merely a low calorie cook book, but a new way of cooking and eating. As he says, no one "is interested in losing weight for a week or a month

and then slowly eating it all back." Thus, he includes some "Dos and Don'ts of Dieting," information about all types of artificial sweeteners, herbs and seasonings without caloric value and general kitchen reference tables. An extensive list of low calorie commercial products called "Groceries for Reducers" was prepared with the co-operation of representatives of the producers of such items.

For the sophisticated homemaker or "amateur chef" with time and luxury facilities at his disposal, following the recipes in this book might become quite a popular indoor sport. The homemaker who has either a diabetic or a reducer in the family, and who can no longer display her creative culinary arts in rich desserts and pastries, will discover in these recipes a new challenge to make special diet meals alluring. Obviously, the help which a homemaker can offer to those of her family or friends on restricted diets is all important in helping them to conform to doctor's orders. The numerous recipes for beverages, soups, main dishes, salads and desserts, as well as the extensive snack suggestions for between meals, each give calories per serving. Generous use is made of herbs, unusual seasoning combinations, skim milk solids and yogurt with very limited use of fats and sugars. Thus, the dishes are suitable for either diabetics or reducers.

Many of the special "Groceries for Reducers," as well as the equipment recommended, would raise the food cost for such a diet above that commensurate with the income of the average or low income family. Moreover, for one who must eat many meals away from home and has little time to prepare his own meals—or is but one in a family which eats regular food—such a book of recipes would be of little help. Nevertheless, there is a place for just such a guide to satisfy the curiosity and urge to do something about this overweight problem, especially among the leisure upper income group.

This book is one, among the numerous reducing books on the market, which seems to offer sound advice and a "do it yourself" challenge to the dieter, which may be the right psychological approach to a group who have found "dieting" beyond their powers of self-discipline. The dedication of this book "To the thin person, inside every fat person, fighting to get out" is truly inspired.

ABSTRACTS

Alella, A.; Williams, F. L.; Bolene-Williams, C.; and Katz, L. N. (Cardiovas. Dept., Med. Res. Inst., Michael Reese Hosp., Chicago, Ill.): INTERRELATION BETWEEN CARDIAC OXYGEN CONSUMPTION AND CORONARY BLOOD FLOW. Am. J. Physiol. *183*:570-82, December 1955.

The determinants of the coronary flow in the anesthetized dog with the heart in situ have been studied under controlled conditions. The following conclusions have been reached.

The coronary flow is not related to the cardiac output. Mean aortic pressure affects the coronary flow but at the same time produces significant changes in oxygen consumption. Within the limits of these experiments, the pure mechanical effect of aortic pressure on coronary flow, independent of its oxygen consumption effect, is slight. Oxygen consumption appears to be the most important factor determining coronary flow. A nomogram showing the relation between coronary flow, mean aortic pressure and oxygen consumption is presented, in which the dominant determining role of oxygen consumption on the quantity of coronary flow is clearly shown. Because of this relationship, it is suggested that, if experimental values of coronary flow are to have meaning, they must first be related to the level of oxygen consumption existing at the moment. With increasing consumption of oxygen, a coronary vasodilatation occurs to meet the oxygen needs of the myocardium. Thus, the availability of oxygen parallels the need for it. An increase in mean aortic pressure produces a slowing of the heart and an increase in coronary flow. However, this does not result in a decreased oxygen consumption by the myocardium.

Alivisatos, John G.; and McCullagh, E. Perry (Cleveland Clin. Foundation and Frank E. Bunts Educational Inst., Cleveland, O.): STABLE AND BRITTLE DIABETES. Am. J. Med. *21*:344-57, September 1956.

In this article, the authors have attempted to determine

clinical and biochemical features to distinguish brittle from stable diabetes and also to determine the incidence of late degenerative complications in these two groups. The patients had been observed for a number of years and were selected during a one-year period; included were those not less than twenty-five years of age who had had not less than sixty blood determinations during several years of follow-up, with an average of fifteen to twenty-five determinations each year.

Of the seventy-nine patients with stable diabetes, forty-five gave type A curves (more complete control of blood sugar levels) and thirty-four gave type B curves (less complete). In seventy patients with brittle diabetes, a v-shaped curve was obtained, which indicated that these patients had a high percentage of blood sugar values at the two extremes, either very low or very high. A greater incidence of insulin shock, a greater incidence of high blood sugars, and a greater incidence of ketonuria further characterized the brittle diabetics. Twenty-three of the patients could not be classified as being either stable or brittle and were an intermediate group. Of the stable diabetics, all were more than forty years of age except two; and of seventy patients with brittle diabetes, fifty-three were more than forty years of age and seventeen between the ages of twenty-five and thirty. Of the stable diabetics, forty-three were women and thirty-six were men. Among the brittle diabetics, forty-four were women and twenty-six were men. Excessive weight was much more common to the stable than to the brittle diabetics. Most brittle diabetics were treated with a mixture of Protamine Zinc and regular insulin. Among brittle diabetics, 60 per cent of the patients had insulin reactions from one to three times weekly. Forty per cent of the stable diabetics never had reactions. Physical exertion led to 86 per cent of the insulin reactions in the brittle diabetic. Twenty-nine per cent of the patients with brittle diabetes had insulogenic lipodystrophy. None was found in patients with stable diabetes.

The incidence of degenerative complications was higher in patients with stable diabetes than in those with brittle diabetes. The incidence of severe diabetic retinopathy in adult patients with long-standing brittle diabetes was very low. Patients with stable diabetes with poor control showed significantly higher incidence of late degenerative complications than patients with stable diabetes with good control. The authors conclude that there are two types of diabetes mellitus, which should be looked upon as distinct metabolic entities.

Ashmore, James; Renold, Albert E.; Nesbett, Frances B.; and Hastings, A. Baird (Dept. of Biol. Chem., Harvard Med. Sch., Boston, Mass.): STUDIES ON CARBOHYDRATE METABOLISM IN RAT LIVER SLICES. V. GLYCEROL METABOLISM IN RELATION TO OTHER SUBSTRATES IN NORMAL AND DIABETIC TISSUE. J. Biol. Chem. *215*: 153-61, July 1955.

The metabolism of C^{14} glycerol in liver slices from normal and diabetic rats was compared with that of glucose, fructose, and pyruvate. All substrates were found to exhibit the same general metabolic pattern. In addition to a diminished utilization of glucose, liver slices from diabetic rats produced more glucose and less glycogen from each of these substrates than did normal liver tissue. The metabolism of liver from diabetic animals could be returned to normal by the injection of insulin prior to sacrifice of the animal.

Barrett, J. L.; Nishikawara, M. T.; and Haist, R. E. (Dept. of Physiol., Univ. of Toronto, Toronto, Ont., Canada): EFFECTS OF HYPOPHYSECTOMY AND OF UNDERNUTRITION ON AMYLOLYTIC ACTIVITY OF THE PANCREAS OF THE RAT. Am. J. Physiol. *182*:35-38, July 1955.

The removal of the pituitary gland was shown to cause a significant decrease in both the concentration of amylase activity and the total amylase activity of the pancreas of the rat. Fasting for a period of 48 to 72 hours in young rats resulted in a reduction in the weight of the pancreas. The amylase activity per unit of pancreas weight was not significantly different from that of control rats freely fed, but the total amylase activity was significantly reduced in the fasted animals. In heavier rats, fasted for a period of 5 or 6 days, pancreas weights, amylase activity per unit weight of pancreas, total activity, and activity per 100 gm. body weight were all reduced significantly. In rats fed an amount of diet just sufficient to maintain body weight, the amylase activity per unit weight of pancreas and the amylase activity per unit of body weight did not differ significantly from the activity in control animals freely fed. The total pancreatic amylase activity was significantly lowered in the animals with restricted caloric intake.

It was concluded that the effect of hypophysectomy on the pancreatic amylase activity could not be ascribed solely to undernutrition and that the pituitary gland exerts a definite influence on the amylase activity of the pancreas of the rat.

Beatty, Clarissa H.; and West, Edward S. (Dept. of Biochem., Univ. of Oregon Med. Sch., Portland, Ore.): EFFECT OF SUCCINIC AND MALIC ACIDS AND FRUCTOSE ON KETOSIS IN ALLOXAN-DIABETIC RATS. J. Biol. Chem. *215*:661-68, August 1955.

Administration of succinic and malic acids was found to have no detectable effect on the ketonuria of alloxan-diabetic rats fed Wesson oil, although the dose was suf-

ficient to increase glucosuria. The administration of small amounts of insulin plus succinic or malic acids caused a larger decrease in ketonuria, in insulin-deficient preparations, than insulin alone. No difference was noted between the effects of glucose and fructose on ketonuria in diabetic ketotic rats. The tricarboxylic acid cycle inhibitor, fluoroacetate, had a greater effect in the diabetic than in control rats. The data are considered as presumptive evidence that insulin may be involved in metabolism at the level of the tricarboxylic acid cycle, possibly in the condensation of acetyl-coenzyme A with oxalacetate.

Bernard, Jack A. (El Paso, Tex.): ORAL HYPOGLYCEMIC AGENTS. Southwestern Med. *37*:588, October 1956.

The author discusses the use of the two oral hypoglycemic agents, namely carbutamide and Orinase. He reports that the prime indication for the use of these drugs is the adult obese type of diabetes and states that the drugs are of little value in the juvenile type of diabetes.

Bloom, Ben (Div. of Nutrition and Physiol., the Pub. Health Res. Inst. of the City of New York, N. Y., and the Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Inst. of Health, Bethesda, Md.): FRACTION OF GLUCOSE CATABOLIZED VIA THE EMBDEN-MEYERHOF PATHWAY: ALLOXAN-DIABETIC AND FASTED RATS. J. Biol. Chem. *215*:467-72, August 1955.

Liver slices obtained from diabetic and from fasted normal rats were incubated with the following substrates: glucose-1-C^{14}, glucose-2-C^{14}, glucose-6-C^{14}, gluconate-1-C^{14}, gluconate-6-C^{14}, lactate-1-C^{14}, and lactate-3-C^{14}. The formation of CO_2 from glucose atoms 1, 2, and 6 was inhibited in diabetes or by fasting, whereas that from gluconate carbons 1 and 6 and lactate carbons 1 and 3 was either normal or augmented. Fatty acid synthesis was strikingly reduced from the substrates investigated. By use of expressions previously presented, the author estimated that the fraction of catabolized glucose molecules which enter the Embden-Meyerhof pathway is increased in diabetes and in fasting.

Bolen, John G. (Ft. Worth, Tex.): DIABETIC CHARCOT JOINTS. Radiology *67*:95-98, July 1956.

The author reports two cases of Charcot joints occurring in diabetic subjects. In each case the foot was involved, and amputation was the necessary treatment.

Burton, Stanley D.; Robbins, Edward D.; Feigenbaum, Lawrence Z.; Friedman, Meyer; Byers, Sanford O.; and Ishida, Tadashi (Harold Brunn Inst. for Cardiovascular Res., Mount Zion Hosp., San Francisco, Calif.): DISCHARGE OF CHOLESTEROL INTO THE PERFUSATE OF ISOLATED RAT LIVERS. Am. J. Physiol. *182*:89-91, July 1955.

The detergent triton (WR-1339), which possesses the unique property of inhibiting the hepatic extraction of cholesterol from the plasma, did not effect significant increases in cholesterol concentration of the perfusate of normal livers, nor did such perfused normal livers show any significant change in cholesterol content or concentration.

These results suggest the isolated liver either is not discharging cholesterol into the perfusate or it is both discharging and taking up cholesterol from the perfusate at approximately equal rates. When perfused with triton, added to the perfusate of livers of hyperthyroid rats, with their accelerated cholesterol metabolism, differentiation between these two alternatives is permitted. The concentration of cholesterol in the perfusate increased by 45 per cent during a six-hour period when triton was added, but no significant use occurred when triton was absent. These results establish that such livers of hyperthyroid rats do discharge cholesterol into the perfusion fluid.

Byers, Sanford O.; and Friedman, Meyer (Harold Brunn Inst. for Cardiovascular Res., Mount Zion Hosp., San Francisco, Calif.): OBSERVATIONS CONCERNING THE PRODUCTION AND EXCRETION OF CHOLESTEROL IN MAMMALS. Am. J. Physiol. *182*:69-72, July 1955.

Simple exclusion of pancreatic flow from the intestine of the rat was found to have little effect upon subsequent cholesterol and lipid absorption. Even when pancreatectomy was added to the procedure, a considerable degree of both cholesterol and lipid absorption still took place. It is believed that major changes in cholesterol absorption cannot be induced by even drastic alteration of pancreatic flow.

Byers, Sanford O.; Rosenman, Ray H.; and Friedman, Meyer (Harold Brunn Inst., Mount Zion Hosp., San Francisco, Calif.): INTESTINAL EXCRETION OF CHOLESTEROL AND TOTAL LIPIDS BY THE NEPHROTIC RAT. Am. J. Physiol. *182*:73-74, July 1955.

The intestinal excretion of cholesterol and total lipids was studied in nephrotic and control rats. Nephrotic and control rats both exhibited the same fecal excretion of cholesterol as well as of total lipids. It was concluded that experimental nephrotic hypercholesteremia and hyperlipemia cannot be ascribed to a diminished intestinal excretion of these substances.

Charles, Bruce (Toronto East Gen. Hosp., and Sunnybrook D.V.A. Hosp., Toronto, Canada): TREATMENT OF DIABETES MELLITUS WITH BZ-55. Canad. M. A. J. *74*:985-87, June 15, 1956.

Four elderly diabetic patients were treated with BZ-55. In two, the insulin requirement was reduced. In one patient the insulin requirement was reduced to zero with

BZ-55, and in one patient there was no effect.

Chase, Lillian A. (Women's College Hosp., Toronto, Ont., Canada): CLINICAL EFFECTS OF BZ-55 (CARBUTAMIDE). Canad. M. A. J. 74:989-90, June 15, 1956.

Two women with diabetes mellitus were treated with BZ-55. In one case some hypoglycemic action was noted, and in the other there was no effect.

Chute, A. L.; and Bain, H. W. (Dept. of Pediat., Univ. of Toronto, and Res. Inst., Hosp. for Sick Children, Toronto, Ont., Canada): EXPERIENCES WITH ORAL SULFONAMIDE (BZ-55) IN THE MANAGEMENT OF JUVENILE DIABETES. Canad. M. A. J. 74:994-97, June 15, 1956.

Five juvenile diabetic patients were treated with BZ-55. Three newly discovered diabetics developed an erythematous rash, and the drug was discontinued. One patient with diabetes of five years' duration failed to show any hypoglycemic effect from the drug. One patient with diabetes of one and a half years' duration showed definite effect of BZ-55 as regards maintenance of relatively normal fasting blood sugar, but there was no significant effect on the postprandial blood sugar levels.

Clarke, W. T. W. (534 Medical Arts Bldg., Toronto, Ont., Canada): CLINICAL EXPERIENCE WITH U-2043 (ORINASE). Canad. M. A. J. 74:998, June 15, 1956.

Six diabetic patients were given U-2043 (Orinase). Three patients in the elderly age group were adequately controlled on the drug. Of three patients in the younger age group, one was controlled adequately and two were not.

Cohen, Julius J.; Berglund, Fredrik; and Lotspeich, William D. (Dept. of Physiol., Univ. of Cincinnati Coll. of Med., Cincinnati, O.): RENAL TUBULAR REABSORPTION OF ACETOACETATE, INORGANIC SULFATE AND INORGANIC PHOSPHATE IN THE DOG AS AFFECTED BY GLUCOSE AND PHLORIZIN. Am. J. Physiol. *184*:91-96, January 1956.

The effects of glucose and phlorizin on the maximal reabsorptive rates of acetoacetate, sulfate and phosphate were studied in the dog. Increased rates of glucose reabsorption depress maximal acetoacetate, sulfate, and phosphate transport. Phlorizin not only reverses this depressant effect of glucose on these three ions but actually raises their maximal reabsorptive rates above those of the control levels. These findings for acetoacetate and sulfate reabsorption are similar to those previously reported for phosphate reabsorption. The three ions, acetoacetate, sulfate, and phosphate, share a common reaction with glucose during their tubular transport. Glucose is the predominant compound, depressing any of the other ions after they have reached maximal reabsorptive rates. Thus the effects of glycosuria on renal conservation of essential elements extend beyond the simple loss of glucose.

Crossland, James; Elliott, K. A. C.; and Pappius, Hanna M. (Donner Lab. of Experimental Neurochemistry, Montreal Neurological Inst., and the Dept. of Neurol. and Neurosurgery, McGill Univ., Montreal, Canada): ACETYLCHOLINE CONTENT OF BRAIN DURING INSULIN HYPOGLYCEMIA. Am. J. Physiol. *183*:32-34, October 1955.

The acetylcholine content of rat brain is decreased during insulin hypoglycemia. This change can be demonstrated only in animals killed by total immersion in liquid air.

Debons, Albert F.; Wallace, John W.; and Bacchus, Habeeb (Dept. of Physiol., George Washington Univ. Sch. of Med., Washington, D.C.): KETONE BODY METABOLISM IN ASCORBIC ACID DEFICIENCY. IN VITRO FORMATION OF KETONE BODIES BY LIVER SLICES. Am. J. Physiol. *185*:31-34, April 1956.

The in vitro formation of ketone bodies by liver tissues of ascorbic acid deficient and control (pair-fed) guinea pigs was studied. The data revealed that the spontaneous production of ketone bodies by the liver of the ascorbic acid deficient guinea pigs is less than that of the control animal. The capacity of the liver of the deficient guinea pig to form ketone bodies from octanoate exceeds that of control animals. The total fat content and the weight are not significantly altered in ascorbic acid deficiency. The data suggest that the diminished spontaneous production of ketone bodies by the ascorbic acid deficient liver, in spite of its normal content of fat, might be secondary to a diminished fatty acid formation from endogenous fat. In the presence of fatty acid substrate, the formation of ketone bodies by the ascorbic acid deficient liver is not appreciably disturbed.

Delle Sedie, P. F.; and Bickel, J. (Ist d'igiene e microbiologia, Universita degli studi, Pisa, Italy): OBSERVATIONS ON THE CARBOHYDRATE METABOLISM OF MYCOBACTERIA AVIARY AND PARATUBERCULOSIS BY MANOMETRIC METHOD. Riv. Ist. sieroterap. ital. *31*:91, March-April 1956.

The carbohydrate metabolism of Mycobacterium tuberculosis var. avium and M. paratuberculosis was studied to evaluate direct respiration by the Warburg method. Striking differences were found in oxidation rate of carbohydrates among the two groups of mycobacteria. The Warburg manometric method has proved to be a valuable and sensitive method. (Italian)

Dickson, H. M. (Dept. of Biology, Brown Univ., Providence, R. I.): EFFECT OF X-IRRADIATION ON GLUCOSE

ABSORPTION. Am. J. Physiol. *182*:477-78, September 1955.

In mice, x-irradiation at a dose level of 500 r. reduced the rate of glucose absorption from the intestine. This effect could not be attributed to retarded gastric emptying or to a diminution of intestinal hexokinase.

Dury, Abraham; and Di Luzio, Nicholas R. (Dorn Lab. for Med. Res., Bradford Hosp., Bradford, Pa.): EFFECTS OF CORTISONE AND EPINEPHRINE EXHIBITION ON LIPID COMPONENTS AND PHOSPHOLIPID TURNOVER IN PLASMA, LIVER AND AORTA OF RABBITS. Am. J. Physiol. *182*:45-50, July 1955.

The concentrations of lipid components and the incorporation of radioactive phosphorus (P^{32}) in the plasma, liver, and aorta of rabbits treated for fourteen consecutive days with single daily injections of epinephrine, cortisone, and cortisone plus epinephrine and in those of control rabbits has been measured. Markedly altered concentrations of lipid fractions in plasma and liver were found in those animals which had received cortisone (alone and in conjunction with epinephrine), and the phospholipids in plasma and liver were synthesized several times faster than in controls. But cortisone exhibition did not change the concentrations of aortic lipid fractions or phospholipid turnover in that organ. Epinephrine exhibition resulted in moderate changes in plasma lipid relationships, and an increased incorporation of P^{32} in the aorta was found.

Emerson, J. D.; and Emerson, G. M. (Dept. of Physiol., Med. Coll. of Alabama and Univ. of Alabama Sch. of Dent., Birmingham, Ala.): FAILURE OF THE RAT TO SHOW CONTINUOUS GROWTH IN RESPONSE TO A CRUDE ALKALINE EXTRACT OF RAT PITUITARY GLANDS. Am. J. Physiol. *182*:521-23, September 1955.

Two seven-month-old female Long-Evans rats were treated for forty days with a growth hormone containing extract of rat pituitary glands. An initial rapid growth was followed by a growth plateau essentially identical to that which has been shown to occur in rats treated with a constant dosage of similar extracts of beef pituitary glands or with purified growth hormone.

An assay was conducted comparing the growth-promoting activity of similar extracts of beef and rat pituitary glands on both young adult female rats and old female rats. The results of this assay indicate that the growth-promoting activities of analogous rat and beef pituitary extracts are of roughly the same magnitude.

Fenton, Paul F. (Dept. of Biology, Brown University, Providence, R. I.): GROWTH AND FAT DEPOSITION IN THE MOUSE: A DEFINITION OF OBESITY. Am. J. Physiol. *184*:52-54, January 1956.

After being weaned, mice of the C_{57}Bl/Fn and I/Fn strain were placed on a diet containing 50 per cent fat. The carcass fat content of C_{57}Bl/Fn mice increased linearly with the age of the animals. Plotting fat content against fat free weight for young animals of the C_{57}Bl/Fn and all animals of the I/Fn strains yielded a straight line of slope 0.1. The data for older C_{57} animals (3 to 5 months) fall on a line of much greater slope. Obesity is defined as a markedly positive deviation from the straight line slope of fat content plotted against fat free weight for young animals.

Field, James B.; and Stetten, DeWitt, Jr. (National Inst. of Health, U.S. Dept. of Health, Education, and Welfare, Bethesda, Md.): HUMORAL INSULIN ANTAGONISM ASSOCIATED WITH DIABETIC ACIDOSIS. Am. J. Med. *21*:339-43, September 1956.

This study was undertaken to determine whether or not humoral insulin antagonism could be demonstrated during acute insulin resistance associated with diabetic acidosis.

By the hemidiaphragm technic of determining, insulin effect was similar to that of Stadie. Each hemidiaphragm from fasted, killed Sprague-Dawley rats was equilibrated, one with a solution containing 1 cc. of serum and 1 cc. of insulin-buffer mixture which contained 0.1 unit per cc., the other with serum alone. The glycogen content of each hemidiaphragm was determined, and differences between those exposed to insulin and their controls were compared for insulin effect. Serum from patients with diabetic acidosis was obtained, and blood levels of compound F were measured. In five of the seven patients with diabetic acidosis, humoral insulin antagonist activity was demonstrated by these technics. Serum of normal subjects depressed insulin little, if at all. Serum from several patients with diabetic acidosis abolished the insulin effect of glycogen accumulation in the rat hemidiaphragm. The insulin antagonist activity was not destroyed by freezing. Neither was it directly attributable to lowered *p*H since the serum of patients in uremic acidosis did not depress the effect of insulin. No inhibition of normal insulin effect could be produced by addition of compound F to normal serum prior to admixture of insulin. The injection of ACTH into a patient with diabetes failed to produce inhibitory serum, even though a rise in circulating compound F occurred. There was no relationship between the level of blood sugar or carbon dioxide on admission and the presence or absence of inhibitor activity. No correlation could be seen with the presence of complications of diabetes, the duration of the disease, or previous insulin therapy. None of the diabetics studied exhibited insulin resistance be-

fore or after the episode of acidosis. Good correlation was seen between the amount of insulin received in the first twenty-four hours of treatment and insulin antagonist activity within the serum. On the basis of this evidence, the lowered *p*H of the blood and the secretions of the adrenal cortex are not regarded as major causative factors in insulin antagonism in diabetic acidosis.

Foà, Piero P.; Galansino, Giorgio; and Costa, Erminio (Dept. of Physiol. and Pharmacol., Chicago Med. Sch., Chicago 12, Ill.): PROLACTIN AND THE SECRETION OF INSULIN AND GLUCAGON BY THE PANCREAS. Am. J. Physiol. *182*:493-96, September 1955.

Eighteen cross-circulation experiments were performed anastomosing the pancreaticoduodenal or mesenteric vein of the donor dog D with a femoral vein of a recipient dog R. The intravenous injection of prolactin into previously untreated normal donors causes a lowering of the blood sugar associated with the appearance of a hypoglycemic material (insulin) in the blood of the pancreatic, but not of the mesenteric vein. This insulin is carried through the anastomosis and causes hypoglycemia in dog R. Prolactin causes hyperglycemia in donor dogs previously treated with repeated injections of this hormone. No hyperglycemic material (glucagon) appears in the pancreatic blood of these animals. These results confirm the hypothesis that the so-called diabetogenic effect of prolactin may be the result of a rapid dumping of preformed insulin by the beta cells of the islets of Langerhans followed by decreased production. The available evidence suggests that prolactin does not cause an increase in glucagon secretion.

Fodden, John H.; and Read, Willard O. (Dept. of Pathol. and Physiol., Univ. of South Dakota Sch. of Med. Sciences, Vermillion, S. D.): ISOLATION OF A HYPERGLYCEMIC, PHOSPHORYLASE-REACTIVATING SUBSTANCE FROM CANINE PANCREATIC BLOOD. Am. J. Physiol. *182*: 513-17, September 1955.

A substance with biological activities similar to those of pancreatic HGF is extractable from canine pancreatic venous blood. Its reactions are basically those of a protein. The amount recovered is increased by pretreatment of the animal with either growth hormone or corticotropin; the condition of alloxan diabetes makes little difference to the total yield. The hyperglycemic activity of this protein is undisturbed by incubation in blood.

Friedman, Meyer; Byers, Sanford O.; and St. George, Shirley (Harold Brunn Inst. for Cardiovas. Res., Mount Zion Hosp., San Francisco, Calif.): DETECTION OF DIETARY CHOLESTEROL-4-C^{14} IN THE HEPATIC RETICULO-ENDOTHELIAL CELL OF THE RAT. Am. J. Physiol. *184*:141-44, January 1956.

Separation and isolation of hepatic parenchymal from reticulo-endothelial cells of rats given cholesterol-4-C^{14} were accomplished by magnetic separation after prior iron administration and phagocytosis. Radioactivity was found in both cell types 6 and 24 hours following oral administration, which indicates that the reticulo-endothelial cells play a role in the disposition of dietary-derived cholesterol.

Frohman, Charles E.; and Orten, James M. (Dept. of Physiol. Chem., Wayne Univ. Coll. of Med., Detroit, Mich.): TRACER STUDIES OF THE ACIDS OF THE TRICARBOXYLIC ACID CYCLE. I. THE FATE OF LABELED ACETATE IN THE LIVERS OF NORMAL AND DIABETIC RATS. J. Biol. Chem. *216*:795-99, October 1955.

The levels and radioactivities of the acids of the tricarboxylic acid cycle were determined in the livers of normal and alloxan-diabetic rats previously injected with acetate-1-C^{14}. The levels and C^{14} activities of the acids were found to be much lower five minutes after the injection in diabetic animals than in normal controls. It is postulated by the authors that the lower counts in the cycle acids from the livers of diabetic animals result from an inhibition of some reaction necessary for the conversion of acetate to citrate.

Fuller, George R.; MacLeod, Martha B.; and Pitts, Robert F. (Dept. of Physiol., Cornell Univ. Med. Coll., New York, N. Y.): INFLUENCE OF ADMINISTRATION OF POTASSIUM SALTS ON THE RENAL TUBULAR REABSORPTION OF BICARBONATE. Am. J. Physiol. *182*:111-18, July 1955.

The effect of potassium infusion on renal tubular reabsorption of bicarbonate has been studied in anesthetized dogs during acute experiments in which the bicarbonate reabsorptive mechanism was kept saturated by intravenous infusion of sodium bicarbonate.

With the establishment of plasma concentrations of potassium up to 12 mEq./L, bicarbonate reabsorption decreased by as much as 1.3 mEq./100 ml. of glomerular filtrate. This effect was seen with both the chloride and bicarbonate salts of potassium. During infusion of potassium chloride or bicarbonate, the excretion of potassium increased about 0.6 mEq./min. for each 1.0 mEq./min. decrease in bicarbonate reabsorption. This reciprocal relationship between potassium excretion and bicarbonate reabsorption is not quantitatively fixed and varies under differing experimental conditions. Diamox (10 mg./kg.) reduced bicarbonate reabsorption to about the same degree as did potassium infusion, but the actions of these two agents were not additive during simultaneous administration. Under the conditions of our experiments,

up to two-thirds of the administered potassium and roughly the same proportion of the administered bicarbonate disappeared from extracellular fluid, presumably through ion exchange with cellular fluids. Our results are consistent with the broad hypothesis that a competitive relationship between secretion of potassium and hydrogen ions by the tubular cells is responsible for the decreased bicarbonate reabsorption and increased potassium excretion observed during potassium infusion. This competition may be based on reciprocal changes in the concentrations of potassium and hydrogen ions in renal tubular cells.

Ginsburg, Jean; and Paton, A. (St. Thomas's Hosp., London, England): EFFECTS IN MAN OF INSULIN HYPOGLYCEMIA AFTER ADRENALECTOMY. J. Physiol. *133*: 59-60, September 27, 1956 (Proc. Physiological Society 20-21, July 1956).

Responses to the hypoglycemia produced by insulin have been studied in twelve patients shortly after bilateral adrenalectomy. The subjects were fasted overnight and were given insulin (0.1 unit/kg. body weight) intravenously. Serial samples of blood sugar, as well as hand and forearm blood flow, were measured by venous occlusion plethysmography. Typical hypoglycemic reactions occurred 30 to 45 minutes after the injection of insulin, corresponding approximately to the lowest level of capillary blood sugar and associated with a marked increase in hand and forearm blood flow. The blood sugar returned to the fasting level at the same rate as it did in an unspecified number of healthy subjects or in the same subject before adrenalectomy. Hand and forearm blood flow were still raised one hour after the injection of insulin.

Hall, W. E.; Little, J. A.; and O'Sullivan, M. O. (Dept. of Med., Univ. of Toronto, and St. Michael's Hosp., Toronto, Ont., Canada): PRELIMINARY EXPERIENCE WITH BZ-55. Canad. M. A. J. 74:991-92, June 15, 1956.

BZ-55 was given to five adult diabetics. It adequately controlled mild diabetes in three patients. A fourth moderately obese elderly patient with mild diabetes remained in good control after BZ-55 was stopped. Failure was reported in one moderately severe elderly diabetic. Adequate blood levels were maintained with 2-gm. doses once daily. No toxic effects were noted.

Harold, Franklin M.; Felts, J. M.; and Chaikoff, I. L. (Dept. of Physiol., Univ. of California Sch. of Med., Berkeley, Calif.): FATE OF CHOLESTEROL-4-C^{14} AND -26-C^{14} IN THE PERFUSED LIVER. Am. J. Physiol. *183*: 459-62, December 1955.

The fate of cholesterol-4-C^{14} and -26-C^{14} in the isolated perfused liver has been studied. Cholesterol-26-C^{14} was readily oxidized to $C^{14}O_2$. Smaller amounts appeared in the bile, both in the neutral and in the acid fractions. Cholesterol-4-C^{14} was not oxidized to $C^{14}O_2$. Instead, the C^{14} was excreted in the bile. About 10 to 20 per cent of the biliary C^{14} was in the neutral fraction; the remainder, in the bile acid fraction. Taurochenodesoxycholic acid, taurocholic acid, and an unidentified substance (compound Y) were shown to be labeled. A time study of the incorporation of C^{14} into these bile acids revealed that taurochenodesoxycholic acid and compound Y became labeled well before any C^{14} appeared in taurocholic acid. Indeed, taurochenodesoxycholic acid is the major product of cholesterol-4-C^{14} metabolism in the perfused liver, presumably because of the gradual breakdown of the organ before taurocholic acid has time to become dominant. The similarity of the results obtained in the present study with those in the intact animal indicates that cholesterol metabolism in the rat may be regarded, qualitatively at least, as the result of processes occurring in the liver.

Hensler, L.; and Hartmann, H. (Dept. of Med. and Patholog., Institute Cantonal Hosp., St. Gall, Switzerland): DIABETES MELLITUS, COMPENSATED BY BETA-CELL ADENOMA OF PANCREAS. Schweiz. med. Wchnschr. *86*:630-31, May 26, 1956.

A 65-year-old woman, whose diabetes was discovered in 1953, developed coronary thrombosis in 1954. When she was placed on 40 units of protamine zinc insulin and diet, her sugar output varied between 0 and 40 grams in 24 hours and her blood sugar was about 200 mg. per cent. Insulin was stopped several months later because of hypoglycemia; and in spite of more carbohydrate in the diet, her urine remained sugar-free and her blood sugar values ranged from 110 to 170. She had to be hospitalized again in 1955 with cerebral hemorrhage; at this time, the blood sugar rose to 280 and later to 360 mg. per cent and the glycosuria to 14 per cent.

At autopsy, besides diffuse arteriosclerosis and cerebral hemorrhage, an adenoma of the pancreas was discovered which histologically proved to contain beta cells almost exclusively. The authors believe that the improvement of the diabetes after the first insulin treatment was due to this beta-cell adenoma.

Ivy, A. C.; Lin, Tsung-Min; and Karvinen, Esko (Dept. of Clin. Sc., Univ. of Illinois Coll. of Med., Chicago, Ill.): ABSORPTION OF DIHYDROCHOLESTEROL AND SOYA STEROLS BY THE RAT'S INTESTINE. Am. J. Physiol. *183*: 79-85, October 1955.

The intestine of the rat has a limited capacity to absorb cholesterol. It amounts to approximately 92 mg.

for a 250-gm. rat, or 370 mg./ kg. body weight per day. This has to be considered in feeding sterols or mixtures of sterols. An increase in the formation and elimination of sterols which do not develop the Liebermann-Burchard color reaction was not observed until cholesterol was added to the basal diet in excess of the capacity of the sterol absorptive mechanism. Dihydrocholesterol, when fed at a level of 192 mg. daily to a 200 to 250-gm. male rat, was absorbed to the extent of approximately 22 per cent. It did not influence the elimination of endogenous sterol. Soya sterols (93 per cent sitosterol), when fed similarly, were also absorbed to the extent of approximately 22 per cent. Evidence of an increase in blood serum sterols was obtained as a result of feeding soya sterols. When 192 mg. of dihydrocholesterol and 96 mg. of cholesterol were fed together, 36 per cent of the cholesterol and 18 per cent of the dihydrocholesterol were absorbed. Since 61 per cent of cholesterol was absorbed when 96 mg. were fed alone, the dihydrocholesterol decreased the absorption of cholesterol by approximately 40 per cent. When a similar mixture of soya sterols and cholesterol was fed, values closely approximating those obtained with dihydrocholesterol were observed. Thus, these sterols decreased the absorption of cholesterol, which accounts for the decrease in the blood cholesterol level when they were fed with cholesterol. Cholesterol, dihydrocholesterol, and soya sterols have approximately the same solubility in oleic acid (22 per cent) and in corn oil (3 per cent), at 38° C. The results indicate that dihydrocholesterol and soya sterols decrease the absorption of cholesterol by competing for the total capacity of the sterol absorptive mechanism on the basis of their relative absorbabilities.

Kalant, N. (McGill Univ. Clin., Royal Victoria Hosp., Montreal, P.Q., Canada): ADRENAL FUNCTION IN ALLOXAN DIABETES. Am. J. Physiol. *182*:503-6, September 1955.

Urinary corticoid excretion of alloxan-diabetic rats was compared with the excretion of normal rats of similar body weight. Fed diabetic animals excreted considerably more of both biologically active and inactive corticoids than did the normals. Fasting had no effect on the excretion by the intact animals but lowered that of the diabetics almost to normal levels. Diabetic rats excreted more of a given dose of exogenous cortisone. In general, there was a high correlation between urine volume and corticoid excretion; this probably indicates dependence of urine corticoid excretion on the urine volume, though the possibility of a specific renal tubular defect in corticoid reabsorption cannot be excluded. In vitro production of corticoids by adrenal glands of diabetic rats was normal, although the glands were somewhat larger. On the basis of these findings, it is felt that the urinary output of corticoids is not necessarily a reliable measure of adrenal activity and that adrenal enlargement may not always indicate increased adrenal function.

Kappeler, R. (Med. Dept. Inselspital, Berne, Switzerland): FAMILIAL HEMOCHROMATOSIS; CASE OF CARDIAC INSUFFICIENCY AND HEMOCHROMATOSIS TREATED BY VENESECTION. Schweiz. med. Wchnschr. *86*:477-81, May 12, 1956.

Three members of a family of six brothers and two sisters showed hemochromatosis. Two of these patients died at the ages of forty-two and forty-four, one of cardiac insufficiency resistant to therapy. The cause of death of the other is not clear. The prognosis of heart failure in hemochromatosis is serious. The third patient has been treated by bleeding. So far, for the first thirteen months, the result of this treatment has been satisfactory.

One of the patients who died had two sons; the other had two sons and one daughter. All four sons, but not the daughter, had an increased iron content of their blood serums but no other signs of hemochromatosis. The surviving hemochromatotic patient has two sons. Only one of them was tested, and his serum showed normal values for iron.

Kimmelstiel, Ruth; and Villee, Claude A. (Dept. of Biol. Chem., Harvard Med. Sch. and Res. Labs. of the Boston Lying-in Hosp., Boston, Mass.): METABOLISM OF C^{14} PYRUVATE BY THE NEWBORN RAT. Am. J. Physiol. *184*:63-68, January 1956.

The authors have studied the enhanced ability of neonatal mammals to survive anoxia. C^{14}-labeled pyruvate was administered intraperitoneally to newborn rats, subsequently subjected to periods of aerobiosis or anaerobiosis. Expired air was analyzed for carbon dioxide; blood, liver, and carcass were analyzed for pyruvate, lactate, glycogen, and total lipids. Small quantities of CO_2 were produced anaerobically, but this was not derived from the direct decarboxylation of pyruvate. Anaerobiosis resulted in a fivefold increase in blood lactate. The liver glycogen content of 10-to-24-hour-old rats treated anaerobically was only 10 per cent of that of those kept in oxygen. The premature and newborn rats were apparently unable to mobilize glycogen in response to anoxia. The amounts of liver or carcass lipids were not significantly different after aerobic or anaerobic conditions. Lipogenesis occurred anaerobically at a rate not greatly different from that observed in aerobic conditions. In contrast, the data suggest that the utilization of lipids is reduced by anaerobiosis. No gross changes were found that could entirely explain the ability of

neonates to survive prolonged oxygen deprivation; but at least part of the energy for survival was provided by accelerated glycolysis.

King, John W.; and Hainline, Adrian, Jr. (Dept. of Clin. Pathology, Cleveland Clin., Cleveland, O.): COMMERCIAL GLUCOSE OXIDASE PREPARATIONS FOR THE DETECTION OF GLUCOSE IN THE URINE. Cleveland Clin. Quart. *23*:212-15, July 1956.

Clinical trial of two commercial glucose oxidase preparations shows them to be more sensitive in the detection of glucose in the urine than the conventional Benedict's test. The glucose oxidase preparations are not as convenient as Benedict's test for large-scale testing; but, if their high sensitivity is taken into consideration, they are excellent for use in the office laboratory, at the bedside, or by the diabetic patient himself. These reagents also are useful as reference tests in the determination of the nature of copper-reducing, nonglucose substances in urine, because they are highly specific for glucose.

Kinsell, Laurance W.; Brown, Frederick R., Jr.; Friskey, Roger W.; and Michaels, George D. (Inst. for Metabolic Research, Highland Alameda County Hosp., Oakland, Calif.): INSULIN-SPARING SULFONAMIDES (Letters to the Editor). J. Clin. Endocrinol. *16*:821-29, June 1956.

The authors report their experience with insulin-sparing sulfonamides in a group of patients. They feel that the probable mechanism of action of these sulfonamide preparations is through exertion of an effect, directly or indirectly, upon carbohydrate metabolism which "extends" the effects of insulin rather than through replacement of insulin. The enzyme level or levels at which this effect is exerted is still unknown. Results of metabolic studies suggest a primary effect upon lipid metabolism or over-all energy requirement. The different patterns of excretion for free and for conjugated sulfonamides suggest different forms of hepatic metabolism of the sulfonamide. The occurrence of neutropenia during heavy dosage with the sulfonamide indicates the need for caution in its use, particularly in view of the lag in complete elimination of sulfonamide from the organism, as indicated by measurable amounts in the blood for some days after the last dose in the patients studied. Investigation in the author's laboratory is being concentrated upon the mechanism of action and upon the use of these agents for possible stabilizing effects in "brittle" diabetics.

Kleeberg, J.; Diengott, D.; and Gottfried, J. (Med. Dept. 'A,' and Dept. of Chemistry, Rothschild Hadassah Univ. Hosp., Jerusalem, and Med. Sch., Hebrew University, Jerusalem, Israel): A CASE OF INSULIN RESISTANCE TREATED WITH CORTICOTROPIN. J. Clin. Endocrinol. *16*:680-86, May 1956.

The authors report a case of insulin resistance which developed in a 62-year-old male with severe diabetes. The diabetes could not be regulated by regular insulin in doses as high as 600 units daily. Administration of ACTH effected a dramatic improvement in a short period of time and permitted a reduction of the daily insulin dose to 80 or 100 units. The marked therapeutic effect of ACTH in this case supports the assumption that the insulin resistance was due to an antigen-antibody reaction. The presence of an anti-insulin factor in a patient's serum could be demonstrated on mice, simultaneously with the presence of resistance to insulin. With the disappearance of the resistance, the insulin-neutralizing factor in the serum also disappeared.

Knowles, Harvey C., Jr.; and Alverson, Gabriele (Children's Hosp. Res. Foundation; and Dept. of Pediat., Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio): THE OSMOTIC ACTIVITY OF THE RENAL TUBULE IN DIABETIC ACIDOSIS. J. Lab. & Clin. Med. *48*:176-83, August 1956.

Observations made on patients with diabetic acidosis revealed no correlation between urinary total osmolarity and flow and the fact that osmotic activity is minimally diminished in the uncomplicated case. The flow is closely related to the total load and to glucose load as well, since glucose comprises the largest constituent of the load and a relatively constant one.

Kornerup, Tore (Ophthalmic Clin., Karolinska sjukhuset, Stockholm, Sweden): STUDIES IN DIABETIC RETINOPATHY: AN INVESTIGATION OF 1.000 CASES OF DIABETES. Acta med. scandinav. *153*:81-101, Dec. 20, 1955.

One thousand unselected diabetics were carefully studied. Diabetic retinopathy was found in 46.8 per cent of the patients. The frequency of diabetic retinopathy increased with duration of the disease, whereas age seemed to have no influence. In diabetics whose disease starts in adult, or advanced age, a relatively early appearance of retinopathy, hypertensive retinal vascular disease, and elevation of blood pressure are to be expected. The systolic and diastolic blood pressures increase with age and also with the duration of diabetes. Fundus hypertonicus in diabetics increases with the age at onset of diabetes but is independent of duration. Diabetic retinopathy and fundus hypertonicus are found to be independent diseases. Proteinuria behaves chiefly like retinopathy, with which it shows a correlation. As a rule, retinopathy precedes the appearance of proteinuria.

Kritchevsky, David; Moyer, A. W.; Tesar, W. C.; Logan, John B.; McCandless, R.F.J.; and Davies, M. C. (Rickett-

sial Sec., Res. Div., American Cyanamid Co., Pearl River, N. Y.): EFFECT OF INJECTED CHOLINE CITRATE IN EXPERIMENTAL ATHEROSCLEROSIS. Am. J. Physiol. *183*: 535-37, December 1955.

Serum cholesterol levels and atheromata in cholesterol-fed rabbits were reduced by intraperitoneal injection of choline citrate but not by choline chloride. Injection of sodium citrate lowered serum cholesterol levels but did not seem to affect the extent of atheromata. None of the three salts used had any effect when administered orally.

Laurell, S. (Dept. of Clin. Chem., Univ. of Lund, Lund, Sweden): PLASMA FREE FATTY ACIDS IN DIABETIC ACIDOSIS AND STARVATION. Scandinav. J. Clin. & Lab. Invest. *8*:81-82, 1956.

Determination of free fatty acids in the plasma in a case of diabetic acidosis revealed an amount greater than normal which was not due to serum lipase activity. The increase was not great enough to account for the observed increased electrophoretic mobility of serum lipoproteins in this condition.

Leibel, B. S. (Diabetic Clin., Sunnybrook D.V.A. Hosp., and Mt. Sinai Hosp., Toronto, Ont., Canada): THE EFFECT OF BZ-55 ON BIOCHEMICALLY UNCONTROLLED DIABETES WITHOUT INSULIN TREATMENT. Canad. M. A. J. 74:979-82, June 15, 1956.

Five patients with uncontrolled diabetes were treated with BZ-55. Of three patients over forty years of age, two were benefited. Of two patients in their thirties, one improved.

Lemley-Stone, Janet; Merrill, Joseph M.; Grace, James T.; and Meneely, George R. (Res. Lab., V.A. Hosp., and the Depts. of Med., Biochem. and Surg., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): TRANSAMINASE IN EXPERIMENTAL MYOCARDIAL INFARCTION. Am. J. Physiol. *183*:555-58, December 1955.

Myocardial infarction was produced by surgical ligation of a branch of the coronary artery in the dog. Following this procedure the serum transaminase activity in nineteen dogs was increased by 355 per cent. Dogs subjected to a sham operation showed slight increases in serum transaminase activity. Evidence was presented which suggested that this elevation was due to a release of transaminase from skeletal muscle damaged by the thoracotomy. The concentration of transaminase was measured in normal and infarcted myocardial tissue; a 31 per cent reduction was found to occur in the infarcted area. If 31 per cent of the enzyme were released from the tissue, each gram of damaged cardiac muscle could supply 122,000 units of transaminase. The size of the infarct was directly proportional to the increased transaminase activity of the serum and inversely proportional to the enzyme concentration of the infarcted myocardial tissue. Animals killed at longer intervals after coronary artery ligation showed both greater elevations in serum transaminase and increasing decrements in transaminase concentration of the infarcted area of the myocardium. On the basis of these findings it was concluded that the major source of the elevated serum transaminase activity after experimental myocardial infarction was the damaged cardiac muscle, although our evidence strongly suggested that injured skeletal muscle and possibly other tissues could contribute in a minor way to the serum enzyme level.

Leonard, Edward; and Orloff, Jack (Lab. of Kidney and Electrolyte Metabolism, Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.): REGULATION OF AMMONIA EXCRETION IN THE RAT. Am. J. Physiol. *182*:131-38, July 1955.

Experiments were performed in an effort to correlate rate of ammonia excretion, alterations in acid-base balance, urine *p*H, and renal glutaminase activity in the rat. The data support the conclusion that renal ammonia production is regulated primarily by factors related to body acid-base changes. The results are in contrast to those obtained in dog and man, in which urine *p*H is a primary factor in the control of both ammonia production and excretion.

The activity of renal glutaminase is elevated in chronically acidotic rats in association with increased rates of urinary ammonia excretion. A rapid rise in ammonia excretion under conditions of acute acid-loading occurs without an appreciable change in glutaminase activity. It is likely that other mechanisms are involved in the augmentation of ammonia excretion under these circumstances.

Lerman, Sidney (Montreal, Que., Canada): DIABETIC RETINOPATHY. Canad. M. A. J. 75:191-93, Aug. 1, 1956.

A review of current views on the etiology and pathogenesis of diabetic retinopathy.

Lin, Tsung-Min; Karvinen, Esko; and Ivy, A. C. (Dept. of Clin. Sci., Univ. of Illinois Coll. of Med., Chicago, Ill.): RELATION OF DIETARY FAT TO THE ABSORPTION AND ELIMINATION OF EXOGENOUS AND ENDOGENOUS CHOLESTEROL. Am. J. Physiol. *183*:86-90, October 1955.

This study was made on male rats weighing 200 to 220 gm. The fats were added to the fat and cholesterol-free diet in the amount of 1 gm., which was 7 per cent by weight of the diet. The daily amount of cholesterol fed amounted to 50 mg. daily. The triglycerides were added in an equimolecular amount of 950 mg. of triolein. Oleic acid definitely and tallow slightly decreased the endogenous elimination of cholesterol, whereas corn oil

and triolein definitely increased it. An increase in indigestible bulk in the form of pectin and protopectin did not change the elimination of endogenous cholesterol. Oleic acid augmented the absorption of exogenous or dietary cholesterol to a greater extent (74 per cent) than did a low-melting-point tallow (39° C.) (59 per cent), corn oil (64 per cent), and triolein (49 per cent), the latter of which had no statistically significant facilitative action as compared with feeding the same amount of cholesterol (50 mg. daily) without any fat (40 per cent). Tripalmitin, trielaidin and palmitic acid actually decreased the absorption of cholesterol, the amounts of absorption being 31, 18, and 20 per cent respectively, as compared with 40 per cent when no fat was fed. Of the 50 mg. of cholesterol fed daily, all but 8, 2, and 3 mg. respectively was recovered in the feces. With triolein being the exception among the fats studied, the percentage of dietary cholesterol absorbed varied directly with the percentage of utilization of the fat fed with the cholesterol. The extent of utilization and the melting point of the fats used and the solubility of cholesterol in them did not adequately explain the differences observed. It is suggested that the results of a study of the ease of esterification and the relative absorbability of the esters of cholesterol may provide the explanation.

Luft, R.; and von Euler, U. S. (Dept. of Endocrinol., Serafimerlasarettet; and Dept. of Physiol., Faculty of Med., Stockholm, Sweden): EFFECT OF INSULIN HYPOGLYCEMIA ON URINARY EXCRETION OF ADRENALINE AND NORADRENALINE IN MAN AFTER HYPOPHYSECTOMY. J. Clin. Endocrinol. *16*:1017-25, August 1956.

In twenty hypophysectomized patients, the daily excretion of adrenaline and noradrenaline was not altered except for some increase during the immediate postoperative period. The intravenous administration of insulin in a dosage of 0.1 i.v. per kg. of body weight caused a hypoglycemia which disappeared more slowly than in normal subjects. The insulin hypoglycemia was accompanied by a significant increase in adrenaline excretion. This demonstrates that the ability to respond to hypoglycemia is not abolished by hypophysectomy.

Insulin hypoglycemia did not induce any significant change in the excretion of noradrenaline.

McCullagh, E. Perry (Cleveland Clin., Cleveland, Ohio): DIABETOGENIC ACTION OF PITUITARY: CLINICAL CONSIDERATIONS. Cleveland Clin. Quart. *23*:47-60, January 1956.

Although the faulty carbohydrate metabolism that characterizes diabetes mellitus is most frequently traceable to dysfunction of the pancreas and resultant deficiency of insulin, other factors also play important roles in carbohydrate metabolism, and their action sometimes causes diabetes or affects an existing diabetes. A comprehensive review is made, from the clinical aspect, of the relationships of pituitary and adrenal factors to carbohydrate metabolism.

McDermott, William V., Jr.; Bartlett, Marshall K.; and Culver, Perry J. (Dept. Surg., Harvard Med. Sch., Massachusetts Gen. Hosp., Boston, Mass.): ACUTE PANCREATITIS AFTER PROLONGED FAST AND SUBSEQUENT SURFEIT. New England J. Med. *254*:379-80, Feb. 23, 1956.

A case is reported in which acute pancreatitis in a healthy, athletic 18-year-old boy followed the sudden ingestion of a huge amount of food after a period of prolonged starvation and dehydration.

MacDonald, Mary K.; and Bhattacharya, S. K. (Dept. of Path. and Clin. Chemistry, Univ. of Edinburgh, Scotland): HISTOLOGICAL CHANGES IN RATS RENDERED HYPERGLYCAEMIC BY INJECTION OF DEHYDROASCORBIC ACID. Quart. J. Exper. Physiol. *41*:153-61, April 1956.

Rats made hyperglycemic by repeated intravenous injections of dehydroascorbic acid (DHA) were killed twenty-four hours (Group 1) and four days (Group 2) after the last DHA injection, and the pancreas, kidneys, liver, adrenals and thyroid were examined histologically. The most marked lesions were found in the islets of Langerhans of the pancreas. In Group 1 animals, there was degranulation and shrinkage of the β-cells, pyknosis of their nuclei, and occasionally vacuolation of their cytoplasm; rarely, actual disintegration of cells was observed. No glycogen was stainable in the β-cells. In Group 2 rats, the islets were small; the β-cells were reduced in numbers, shrunken, and usually agranular, with pyknotic nuclei. Cytoplasmic vacuolation was obvious; and although some glycogen was demonstrable in the cells, all the vacuolation could not be accounted for in this way. The kidneys showed no significant degenerative change in either group, but there was some glycogen storage in the tubular cells of Group 2 rats. No change of note was found in the livers or thyroids of the experimental animals.

Mallov, Samuel; and Bloch, Janet L. (Dept. of Pharmacol., State University of New York, Upstate Med. Center, Syracuse, N. Y.): ROLE OF HYPOPHYSIS AND ADRENALS IN FATTY INFILTRATION OF LIVER RESULTING FROM ACUTE ETHANOL INTOXICATION. Am. J. Physiol. *184*:29-34, January 1956.

Acute ethanol intoxication was found to promote fatty infiltration of the liver in rats. The liver lipid concentrations gradually rose to peak values in 15 to 19 hours,

then slowly returned to normal after thirty-six hours. The duration of the fatty infiltration and the peak liver lipid values obtained were functions of the dose of ethanol administered. Under the same conditions, female rats showed a more severe fatty infiltration than did males. The prior administration of large quantities of choline reduced the intensity of the fatty infiltration provoked by the ethanol. In contrast to intact animals, neither adrenalectomized nor hypophysectomized rats showed an accumulation of liver lipids as a result of acute ethanol intoxication. Adrenalectomized rats maintained on cortisone and adrenal-demedullated rats, however, showed the same liver lipid response to ethanol as did intact rats. Rats chronically intoxicated for a period of thirty days exhibited hypertrophy of the adrenals. Acute intoxication produced by isopropanol administration also resulted in the accumulation of liver lipid. It is suggested that ethanol intoxication may cause the mobilization of fat from the depots to the liver and that pituitary and adrenal cortical hormones are involved in the mechanism of this mobilization.

Marques, Maria (Inst. of Exper. Physiol., Porto Alegre Med. Sch., Brazil): DIABETOGENIC ACTION OF GROWTH HORMONE IN THE PHRYNOPS HILARII TURTLE. Rev. Soc. argent. biol. *31*:177, August 1955.

The administration of growth hormone in amounts of 0.25, 0.5, 1, and 5 mg. per kg. daily produced transient diabetes in the Phrynops hilarii after subtotal pancreatectomy. During the induced hyperglycemia, there were lesions of the beta cells: degranulation, vacuolization, pyknosis, or necrosis. When hyperglycemia disappeared, the cells recovered their normal appearance. The nonpancreatectomized turtles showed a marked sensitivity to the diabetogenic action of the growth hormone.

Mayer, Jean; French, Rosalie G.; Zighera, Claudine F.; and Barrnett, Russell J. (Dept. of Nutrition, Harvard Sch. of Pub. Health and the Dept. of Anat., Harvard Med. Sch., Boston, Mass.): HYPOTHALAMIC OBESITY IN THE MOUSE: PRODUCTION, DESCRIPTION AND METABOLIC CHARACTERISTICS. Am. J. Physiol. *182*:75-82, July 1955.

Hypothalamic obesity has been induced in Swiss mice as well as in thin littermates of mice with the hereditary obese-hyperglycemic syndrome. A modification of a previous stereotaxic instrument designed for rats (Krieg) which permits the placing of reproducible lesions in the mouse's brain is described. Co-ordinates of successful lesions are given. Successful lesions involved both ventromedian nuclei. A nearby portion of the hypothalamus was also involved in most animals. Besides obesity, ovarian atrophy was noted as a result of these lesions. In Swiss mice with hypothalamic obesity, the positive energy balance is the result of both marked hyperphagia and very low spontaneous activity. Oxygen consumption is proportional to surface area and of equivalent magnitude to that of nonoperated Swiss mice.

Swiss mice with hypothalamic obesity and "nonobese" littermates of hereditarily obese mice with induced hypothalamic obesity both exhibit similar metabolic characteristics, such as normal blood glucose levels, resistance to the diabetogenic action of growth hormone, and normal sensitivity to the hypoglycemic effect of insulin.

Weight gain was greatest on high-fat diets and minimal on high-protein diets. The fact that genetic, hypothalamic, and goldthioglucose obesity can be either found or produced in littermate animals offers a useful tool for the study of experimental obesity. Comparisons of three types of obese mice which differ in the relative importance of various factors in the establishment of a positive energy balance emphasize the multiplicity of mechanisms leading to obesity.

Mayer, Jean; and Yannoni, Claudine Z. (Dept. of Nutrition, Harvard Sch. of Pub. Health, Boston, Mass.): INCREASED INTESTINAL ABSORPTION OF GLUCOSE IN THREE FORMS OF OBESITY IN THE MOUSE. Am. J. Physiol. *185*:49-53, April 1956.

In three types of obesity in the mouse—obese-hyperglycemic syndrome, goldthioglucose obesity, and hypothalamic obesity—an increase in intestinal absorption of glucose is observed when the dose administered is sufficient. The difference with normal animals is due neither to differences in gastrointestinal weight nor to differences in rate of gastric emptying. This phenomenon appears to be an adaptative result of prolonged hyperphagia.

Metzler, W. S. (Med. Dept., St. Joseph's Hosp., Toronto, Ont., Canada): TREATMENT OF DIABETES MELLITUS WITH BZ-55. Canad. M. A. J. 74:987-88, June 15, 1956.

Three diabetic patients were administered BZ-55. In the first, insulin requirement was reduced to zero, and it was felt that the patient's diabetes might well have been controlled with diet alone. In the second, a juvenile with diabetes of considerable severity, no effect was obtained. In the third, an elderly male, the insulin requirement was reduced to zero.

Moschcowitz, Eli (Dept. of Path., Mount Sinai Hosp., New York, N. Y.): THE PATHOGENESIS OF THE HYALINIZATION OF THE ISLANDS OF LANGERHANS. A.M.A. Arch. Path. *61*:136-42, February 1956.

The island lesions in adult diabetes mellitus are nearly always associated with arteriosclerosis of the pan-

creatic vessels and may be interpreted as a capillary sclerosis, precisely comparable to that found in the alveolar capillaries of the lung and the sinusoids of the liver and in the glomeruli in the presence of arteriosclerosis or phlebosclerosis affecting the main supplying vessels of these organs. The capillary sclerosis is the result either of the extension of the fibrosing or hyalinizing lesion from the afferent arteriole of the island into the capillaries or of a diminution of the blood supply. Island lesions in adult diabetes occur in a little less than half of the cases. It does not occur more frequently because of the special freely anastomosing pattern of both the grosser and the capillary blood supply of the pancreas and of the islands. The cause of the hypoinsulinemia in the cases in which the islands are intact is ascribable to an insufficiency, consequent to an impairment of the grosser blood supply. In juvenile diabetes, in which island lesions occur very rarely if at all, the cause of the diabetes cannot be explained anatomically.

Müller, F. (Dept. of Ophthalmology, Univ. of Leipzig, Germany): ABNORMALITIES OF THE EYE IN DIABETES. Deutsche med. Wchnschr. *81*:941-44, June 8, 1956.

Paralysis of the extrinsic and intrinsic muscles of the eye is relatively rare in diabetes mellitus, as is retrobulbar neuritis. Conjunctiva and iris, however, often show abnormal blood vessels. There is an increased tendency towards glaucoma and iritis. Changes in the lens, especially acutely precipitating or slowly progressive opacities, are at least partially related to disturbances of water and electrolyte balance. Hypotonia of the eyeballs, seen in diabetic coma, is caused by dehydration. The most frequent abnormality is diabetic retinopathy. Three types are distinguished: (1) diabetic retinitis, with aneurysms of capillaries, punctate hemorrhages, and white exudates, especially at the posterior pole; (2) arteriosclerotic retinopathy similar to and indistinguishable from true arteriosclerotic retinitis and that seen in hypertension—however, it occurs much more frequently and earlier in diabetics; and (3) retinitis proliferans, with marked aneurysmal and varicose changes, vessel proliferation, and thrombotic processes. Hemorrhages into the vitreous humour and secondary glaucoma may result. There is apparently a relationship between this type and the changes seen in glomerulosclerosis. All diabetic retinopathies occur independently of age but are more frequent the longer the duration of diabetes. Qualitative changes of blood lipids may play a role in pathogenesis. Apart from good control of diabetes, treatment is symptomatic and, on the whole, unsatisfactory.

Nakada, Henry I. (Scripps Metabolic Clin., La Jolla, Calif.): THE METABOLISM OF FRUCTOSE BY ISOLATED RAT DIAPHRAGMS. J. Biol. Chem. *219*:319-26, March 1956.

Data are presented which confirm the findings of other workers that the uptake of fructose by the rat diaphragm is increased in the presence of insulin. A small but consistent increase in fructose oxidation due to insulin was also noted. In the presence of glucose or glucosamine, the oxidation of fructose by the diaphragm was reduced considerably. The presence of glucose reduced the uptake of fructose by about one-half. The formation of glycogen from fructose was considerably depressed in the presence of glucose. Acid hydrolysis of the hexose monophosphate indicated that, in the presence of glucose, fructose forms an acid-labile phosphate ester which is believed to be fructose-1-phosphate. These findings are considered to be confirmatory of the findings of other workers that muscle cells do not readily utilize fructose per se.

Nakada, Henry I.; Morita, Toshiko N.; and Wick, Arne N. (Scripps Metabolic Clin., La Jolla, Calif.): STUDIES ON THE RELATIONSHIPS BETWEEN INSULIN, GLUCOSAMINE, AND GLUCOSE IN RAT DIAPHRAGMS. J. Biol. Chem. *215*:803-08, August 1955.

Insulin was found to increase the disappearance of glucosamine when incubated with isolated rat diaphragm. Glucosamine inhibited the uptake of glucose by the rat diaphragm. Glucose oxidation was decreased more by glucosamine than by fructose or ammonium chloride when these were incubated with rat diaphragm. The authors believe that these results can be interpreted as a competitive action between glucosamine and glucose for transport into the cell, the suggested mechanism of insulin action.

Nakada, Henry I.; and Wick, Arne N. (Scripps Clin. and Res. Foundation, La Jolla, Calif.): GALACTOSE METABOLISM BY THE ISOLATED RAT DIAPHRAGM. Am. J. Physiol. *185*:23-26, April 1956.

Insulin-sensitive uptake of galactose by the isolated rat diaphragm has been confirmed. Substrate competition studies using radiocarbon-labeled sugars indicate that galactose has no effect on either the uptake or oxidation of glucose. Glucose lowers galactose uptake slightly and reduces its oxidation by 25 per cent. Glucose drastically depresses the oxidation and uptake of fructose, whereas galactose has a negligible effect on it. The distribution of C^{14} in the diaphragm from glucose and galactose was compared. Glucose-C^{14} activity was found in the metabolic products, such as glycogen, CO_2, organic phosphates, and other products. Most of the radioactive galac-

tose was found in the free form. The small amounts of radioactivity found in the respired CO_2, glycogen, and organic phosphates do indicate that galactose can be metabolized by the muscle cells.

Narahara, H. T.; Tomizawa, Henry H.; Miller, Ruth; and Williams, Robert H. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): INTRACELLULAR DISTRIBUTION OF AN INSULIN-INACTIVATING SYSTEM OF LIVER. J. Biol. Chem. *217*:675-84, December 1955.

The authors used insulin labeled with I^{131} for assaying the degradation of insulin by rat liver homogenates. Investigation of the intracellular distribution of the heat-labile insulin-inactivating system in rat liver homogenate after differential centrifugation showed that most of the activity was found in the residual sucrose supernatant fraction.

Ogryzlo, M. A.; and Harrison, Joan (Dept. of Med., Univ. of Toronto, and Clin. Invest. Unit, Sunnybrook Hosp., Toronto, Ont., Canada): THE EFFECT OF BZ-55 (CARBUTAMIDE) ON PANCREATIC DIABETES FOLLOWING PANCREATECTOMY. Canad. M. A. J. *74*:977-78, June 15, 1956.

BZ-55 was not effective in improving control or lowering insulin requirement in patient with diabetes following pancreatectomy.

Park, C. R.; Bornstein, J.; and Post, R. L. (Dept. of Physiol., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): EFFECT OF INSULIN ON FREE GLUCOSE CONTENT OF RAT DIAPHRAGM IN VITRO. Am. J. Physiol. *182*:12-16, July 1955.

Insulin increases the free glucose content of the isolated rat diaphragm when the muscle is incubated in a very high concentration of glucose or at low temperature. Studies with C^{14}-labeled glucose indicate that insulin does not increase glucose production by the diaphragm. The increase in the glucose content of the tissue in the presence of insulin, therefore, arises from glucose which has penetrated from the external medium. It is concluded that an action of insulin on glucose uptake is to accelerate a step concerned with the transfer of glucose into the tissue. This step antecedes and is distinct from glucose phosphorylation by the hexokinase reaction.

Park, C. R.; and Johnson, L. H. (Dept. of Physiol., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): EFFECT OF INSULIN ON TRANSPORT OF GLUCOSE AND GALACTOSE INTO CELLS OF RAT MUSCLE AND BRAIN. Am. J. Physiol. *182*:17-23, July 1955.

Normal, eviscerated, and alloxan-diabetic rats received glucose by continuous intravenous infusion with or without added insulin. In all cases, insulin caused a marked rise in the free glucose content of the diaphragm and heart. In similar experiments, eviscerated rats were infused with galactose. Insulin caused a marked rise in the free galactose content of diaphragm, heart, and gastrocnemius. In muscle, glucose and galactose were extracellular in the absence of insulin but were largely intracellular in the presence of insulin. In brain, free intracellular galactose was found in equally large amounts in the presence or absence of insulin. These findings have been interpreted as follows: (a) Transport of glucose across the cell membrane is the rate-limiting step for glucose uptake (and utilization) by muscle. (b) The transport of glucose and galactose is accelerated by insulin. (c) The transport is inhibited in alloxan-diabetic muscle and can be accelerated by insulin. (d) The transport process in muscle is prior to and distinct from the hexokinase reaction. (e) The transport of galactose, and probably that of glucose, into the cells of brain tissue is not accelerated by insulin.

Peden, J. C., Jr.; Pareira, M. D.; Bond, L.; and Riley, J. S. (Surg. Metabolism Div., Homer G. Phillips Hosp.; Dept. of Surgery, Washington Univ. Sch. of Med., St. Louis, Mo.): COMPARATIVE UTILIZATION OF INTRAVENOUS FRUCTOSE AND INVERT SUGAR. Gastroenterology *30*:804-12, May 1956.

The authors studied the total twenty-four-hour urinary losses of sugar following infusion of 10 per cent fructose and 10 per cent invert sugar in normal subjects at rates of 1,000 ml. per hour or 1,000 ml. per 2.5 hours on alternate days; each patient thus served as his own control. The average losses were 3.8 per cent to 7.1 per cent of the total amount infused, with a maximum of 11.4 per cent in any single patient. The results of this study suggest that 10 per cent invert sugar is not utilized as though the 5 per cent fructose and 5 per cent glucose components are treated independently by the body, but rather that the urinary sugar losses lie between those anticipated with 10 per cent fructose alone and 10 per cent glucose alone. There is certainly no suggestion of a favorable effect of one sugar on the other, and retention of fructose is even less than would be expected had it been given alone. After major operations, infusions of both fructose and invert sugar are efficiently retained, just as in the case of normal patients. One patient excreted an unusual amount of glucose in the urine after infusions of fructose. Postoperative urinary losses of sodium and potassium are of the order noted in previous studies with glucose infusion or with no infusion. Average losses of sodium were slightly higher with fructose than with invert sugar, but in neither case is the loss exceptional (56.9 mEq. and 39.8 mEq.).

Pedersen, Jørgen (Fødeafdeling B, Rigshospitalet, Copenhagen, Denmark): DIABETES AND PREGNANCY. Nord. med. 56:1367-71, Sept. 20, 1956.

The long-term treatment of pregnant diabetics in Maternity Department B, Rigshospitalet, Copenhagen, is described. In 142 cases treated accordingly during the years 1946-1955, the fetal mortality was 11 per cent; whereas in a control group (short-term treated) comprising 144 cases, it was 33 per cent.

A low fetal mortality presupposes intensive medical treatment during pregnancy and the service of expert obstetricians. It is not necessary to give sex endocrine therapy. The rate of cesarean sections was not high.

The author's treatment may be characterized as prophylactic, the effect of which is to lower the maternal complications during pregnancy and to make the infants more normal, especially as regards neonatal blood sugar, weight and length, volume of liquor amnii, and fetal mortality.

Perry, W. F.; and Bowen, Helen F. (Dept. of Physiol. and Med. Res., Univ. of Manitoba, Winnipeg, Canada): EFFECT OF ADRENALECTOMY ON THE INCORPORATION OF ACETATE INTO FATTY ACIDS, CHOLESTEROL AND ACETOACETIC ACID BY RAT LIVER SLICES. Am. J. Physiol. *184*:59-62, January 1956.

The incorporation of C^{14}-acetate into fatty acids, cholesterol, acetoacetic acid, and CO_2 by liver slices of intact and adrenalectomized rats was studied. The slices were incubated in bicarbonate and phosphate buffers. It was found that, in both buffer systems, incorporation into fatty acids and cholesterol was depressed but incorporation into acetoacetic acid was unaffected by adrenalectomy. However, total acetoacetic acid formation by the slices tended to be higher in preparations from adrenalectomized animals. The amount of acetate carbon which appeared as CO_2 was similar with slices from both types of animals. Bicarbonate was found to be a more favorable medium than PO_4 for fatty acid formation, whereas PO_4 was the more favorable medium for cholesterogenesis, though the differences between adrenalectomized and intact animals persisted in either buffer. Forced feeding with glucose increased the incorporation of acetate into fat and cholesterol in both buffer systems in adrenalectomized as well as in intact animals. However, the adrenalectomized preparation still incorporated acetate to a lesser extent than did the controls. It was concluded that, although the depressed incorporation of acetate into fatty acid and cholesterol by liver slices from adrenalectomized animals was consistent with a depressed synthesis of these lipids, there was also the possibility that it reflected an increased turnover of lipids in the liver of the adrenalectomized animal.

Pincus, I. J.; Grice, M. S.; Dunn, M.; and Rutman, J. Z. (Dept. of Physiol., Jefferson Med. Coll., Philadelphia, Pa.): EFFECT OF GLUCAGON (HGF) ON DEPOSITION OF MUSCLE GLYCOGEN. Am. J. Physiol. *183*: 413-15, December 1955.

It has been demonstrated that glucagon (HGF) has a peripheral as well as an hepatic effect on glucose utilization. It inhibits the formation of muscle glycogen produced by insulin.

Glucagon, which is protein in nature, appears to be another hormone acting on a single enzyme system or a group and affecting both hepatic and peripheral glucose metabolism.

Rabson, S. M.; and Mendenhall, E. N. (Depts. of Path. and Gen. Practice, St. Joseph Hosp., Fort Wayne, Ind.): FAMILIAL HYPERTROPHY OF PINEAL BODY, HYPERPLASIA OF ADRENAL CORTEX AND DIABETES MELLITUS: REPORT OF 3 CASES. Am. J. Clin. Path. *26*:283-90, March 1956.

Two sisters and a brother developed a syndrome of pineal body hypertrophy, secondary sexual precocity, and diabetes mellitus at about the same period of life. They all died at approximately the same age. It is suggested that familial pineal deficiency (hypertrophy was evidence of the attempt to overcome the defect) stimulated the anterior lobe of the pituitary body and the adrenal cortex. The one produced external precocity and the other intractable diabetes.

Rall, T. W.; Sutherland, Earl W.; and Wosilait, Walter D. (Dept. of Pharmacol., Sch. of Med., Western Reserve Univ., Cleveland, Ohio): THE RELATIONSHIP OF EPINEPHRINE AND GLUCAGON TO LIVER PHOSPHORYLASE. III. REACTIVATION OF LIVER PHOSPHORYLASE IN SLICES AND IN EXTRACTS. J. Biol. Chem. *218*:483-95, January 1956.

In the second paper, the authors presented evidence which showed that the enzymatic inactivation of liver phosphorylase was accompanied by the removal of phosphate from the enzyme. On the basis that the conversion of dephosphophosphorylase (inactive liver phosphorylase) to the active enzyme should involve the addition of phosphate to the molecule, the authors studied the reactivation process in liver slices with P^{32}-orthophosphate, to detect entrance of phosphate into the enzyme, and also in liver extracts with purified dephospho-liver phosphorylase as substrate. It was found that P^{32}-orthophosphate was rapidly converted into phosphorylase in liver slices; this incorporation was greatly increased by epinephrine and glucagon. Phosphate transfer to dephosphophosphorylase was studied in broken-cell preparations. A soluble liver enzyme, liver dephosphophosphorylase kinase, capable of converting liver dephos-

phophosphorylase to liver phosphorylase in the presence of ATP and magnesium ions was found and purified.

Rauschkolb, Elizabeth W.; Farrell, Gordon L.; and Koletsky, Simon (Dept. of Physiol. and Pathol., Western Reserve Univ. Sch. of Med., Cleveland, O.): ALDOSTERONE SECRETION AFTER HYPOPHYSECTOMY. Am. J. Physiol. *184*:55-58, January 1956.

Adrenal venous blood was collected from hypophysectomized and sham-hypophysectomized dogs six days postoperatively. 17-hydroxycorticosterone, corticosterone, 11-desoxy-17-hydroxycorticosterone, and aldosterone were isolated by paper chromatography. Histologic examination of the sellar and suprasellar regions of the hypophysectomized dogs demonstrated the absence of pituitary tissue. The adrenal glands of the hypophysectomized dogs showed cortical atrophy which did not involve the zona glomerulosa. The rate of secretion of aldosterone by the hypophysectomized dogs was found to be approximately 66 per cent of that of the control (sham-hypophysectomized) dogs. The rates of secretion of 17-hydroxycorticosterone, corticosterone, and 11-desoxy-17-hydroxycorticosterone were found to be approximately 10 per cent of that of the controls. The ability of the hypophysectomized dog to remain in electrolyte balance appears to be due in large measure to the continued secretion of aldosterone.

von Rechenberg, H. K. (Medical Policlinic, University Basel, Switzerland): DIABETES THERAPY IN TODAY'S PRACTICE. Schweiz. med. Wchnschr. *86*:274-79, March 17, 1956.

Review of the treatment used at the medical policlinic in Basel. (German)

Robbers, Hans; and Speck, Friedl (Fürst-Carl Landes-Krankenhaus, Sigmaringen, Germany): THE TREATMENT OF DIABETES MELLITUS WITH NADISAN (BZ-55 OR CARBUTAMIDE). Deutsche med. Wchnschr. *81*:1278-80, Aug. 10, 1956.

Best results in the treatment of diabetes with N-1-sulfanilyl-N-2-butyl-carbamide (BZ-55 or carbutamide) were obtained in over two-thirds of the authors' own patients over the age of fifty (53 of 74 patients). Successes in treatment decreased with decreasing age. Other factors influencing results were duration of the disease and duration of insulin administration before Nadisan was first given. Good results are not to be expected in patients under the age of thirty. In general, administration of Nadisan either gave excellent results or showed no effect at all. Alleged partial successes are probably due to better dietary control during hospitalization. Side-effects included fatigue, erythema, and papular skin eruptions, which made it necessary to discontinue treatment in six patients. In two other cases, administration could be continued despite some side effects. The authors recommend that Nadisan be tried in all diabetics, whatever their age, because the response of any one patient cannot be predicted. However, if Nadisan is given to juvenile diabetics, it should first be administered with insulin, which may be decreased gradually according to need. In this manner, possible coma in juvenile diabetics can be avoided.

Robertson, S. E. J. (Sydney, Australia): SOME ASPECTS OF DIABETES MELLITUS IN CHILDHOOD. M. J. Australia *1*:218-20, Feb. 11, 1956.

A series of patients in a diabetic clinic in a large children's hospital has been studied for a period of two years. A brief summary of the management of diabetic coma is made. Methods of bringing the child under control and maintaining this state by means of insulin and diet are discussed. It is emphasized that day-to-day alterations in insulin doses are necessary for successful control of diabetes in children, and this is best obtained by education of the parents. Various forms of insulin therapy have been tried, and it has been found that the most successful results have been obtained by the use of insulin-zinc suspensions. Brief mention is made of insulin atrophy and the necessity for avoiding severe hypoglycemia in children.

Satke-Eichler, Inge; and Stumpf, Ch. (University of Vienna, Vienna, Austria): DELAYED CONVULSION-EFFECT OF HIGHER INSULIN DOSES IN ADRENALECTOMIZED MICE. Acta endocrinol. *21*:299-304, March 1956.

It has been shown that, in normal and adrenalectomized mice, the period of time between the injection and the onset of the convulsions decreases within a dosage range of 0.0128 I.U. to 1.6 I.U. of insulin/10 gm. body weight, whereas it increases when the insulin dose is raised to 40 I.U./10 gm. body weight. Therefore, the long duration of the period of latency after application of extremely high doses of insulin cannot be due to the action of insulin-antagonizing hormones of the adrenals.

Schumacher, H.; and Schumacher, J. (Medizingeschichtlichen Institut der Universität Freiburg i. Br., Germany): THEN AND NOW: 100 YEARS OF DIABETES MELLITUS. München. med. Wchnschr. *98*:601-04, April 27, 1956.

The author points out that current concepts of diabetes mellitus are still not precisely established. The author attempts to give a general survey of the development of the present concepts of this disease. Various theories on causal mechanisms based on endocrine,

humoral, and central-nervous-system processes are discussed, as they were developed by scientific research during the last century. (German)

Schumaker, Verne Norman (Donner Lab. and the Radiation Lab., Univ. of California, Berkeley, Calif.): CHOLESTEROLEMIC RABBIT LIPOPROTEINS: SERUM LIPOPROTEINS OF THE CHOLESTEROLEMIC RABBIT. Am. J. Physiol. *184*:35-42, January 1956.

The chemical composition in rabbits of the low-density lipoproteins (S_f 0-25, S_f 15-100, and S_f 20-400) has been investigated. Quantitative protein determinations were also made upon each group of lipoproteins. The molecules are shown to contain 40 to 60 per cent cholesterol esters, 10 to 20 per cent unesterified cholesterol, 10 to 20 per cent phospholipid, and 3 to 30 per cent protein, depending upon the sedimentation characteristics. The quantity of glyceride was usually less than 10 per cent for all of the above-mentioned groups of lipoproteins. The quantity of unesterified fatty acids was always less than 6 per cent. Two of the four animals from which samples were withdrawn and analyzed had previously received daily heparin injections for a period of ten weeks.

The effects of heparin injections upon the ultracentrifugal patterns of a larger group of rabbits were also studied. No significant changes in the concentrations of the S_f 5-15, S_f 15-40, and S_f 40-100 species of lipoproteins were found which could be attributed to the heparin injections. The major peak, usually to be found in the S_f 5-15 region, did show a small but significant decrease in sedimentation coefficient in response to heparin injection. Serum from all of the animals receiving heparin did show a definite lipolytic activity in vitro, as measured by transmission values in the egg liproprotein test.

Since the lipemia-clearing activity of heparin appears to be due to lysis of neutral fat glycerides, their small concentration in cholesterolemic rabbit serum probably explains the failure of heparin to produce a significant shift in the lipoprotein spectrum.

Shore, M. L.; Zilversmit, D. B.; and Ackerman, R. F. (Divs. of Physiol., Preventive Med. and Med., Univ. of Tennessee, Memphis, Tenn.): PLASMA PHOSPHOLIPIDE DEPOSITION AND AORTIC PHOSPHOLIPIDE SYNTHESIS IN EXPERIMENTAL ATHEROSCLEROSIS. Am. J. Physiol. *181*:527-31, June 1955.

The authors investigated the role of plasma phospholipids in the development of experimental rabbit atheromatosis by the use of P^{32}-labeled material. Present results indicate that deposition of plasma phospholipids accounts for one-tenth or less of the phospholipid present in the atheromatous thoracic aorta. The data presented support the view that the major portion of aortic phospholipid in experimental atheromatosis is synthesized by the aorta.

Shrifter, Norman; and Kritzer, Morton D. (Los Angeles County Gen. Hosp.; Univ. of Southern California Sch. of Med., Los Angeles, Calif.): CARBOHYDRATE METABOLISM: II. CHANGES IN THE SERUM PYRUVIC ACID DURING GLUCOSE TOLERANCE TEST IN NORMALS, DIABETICS, AND PREDIABETIC WOMEN. A.M.A. Arch. Int. Med. *98*:28-34, July 1956.

Following the oral administration of glucose, normal and prediabetic subjects show a prompt rise in the blood level of pyruvic acid followed by a gradual fall. In the person with long-standing mild diabetes, the blood pyruvate rises gradually during the entire procedure; the recent diabetic exhibits a similar slow steady rise, but with a terminal drop. During the intravenous glucose tolerance test in normal persons, the blood pyruvic acid roughly parallels the blood sugar; whereas in the diabetic, the pyruvate first decreases, then increases, and then falls again. It is suggested that the early drop in pyruvate following intravenous glucose in the diabetic may be due to retarded glycolysis.

Shuman, Charles R.; Kemp, Robert L.; Coyne, Richard; and Wohl, Michael G. (Temple Univ. Hosp. and Metabolic Div., Philadelphia Gen'l. Hosp., Philadelphia, Pa.): CLINICAL USE OF SORBITOL AS A SWEETENING AGENT IN DIABETES MELLITUS. Am. J. Clin. Nutrition *4*:61-67, January-February 1956.

A palatable "ice cream" preparation, in which sorbitol has been used as the sweetening agent in place of sucrose, was fed to thirty-eight diabetic patients. The slow absorption of sorbitol and its conversion to fructose and glycogen prior to its availability as glucose have led to consideration of this substance for use as an adjunct in diabetic diets. In mild and moderately severe diabetic patients, the feeding of sorbitol ice cream as an addition to the usual diet did not significantly alter the diurnal blood glucose values. If further studies support these findings, it is possible that sorbitol ice cream may be permitted in the diet of the diabetic without substitution for its caloric equivalent. At present, sorbitol may be regarded as an available carbohydrate until additional studies further delineate its position in human metabolism and in the diabetic patient.

Silvis, Capt. Richard S.; Simon, Lt. Daniel S. (United States Naval Hosp., Oakland, Calif.): MARKED HYPOGLYCEMIA ASSOCIATED WITH NON-PANCREATIC TUMORS. New England J. Med. *254*:14-17, Jan. 5, 1956.

The literature is reviewed, and the five previously reported cases of hypoglycemia associated with non-

pancreatic tumors are summarized. A case of severe hypoglycemia associated with a large retroperitoneal fibroma is presented. Removal of the tumor cured the hypoglycemia. Possible causes for the altered physiology are discussed. An unknown mechanism for the control of blood sugar may be involved.

Sirek, Anna; and Sirek, Otakar V. (Banting & Best Dept. Med. Res., Univ. of Toronto, and Res. Inst. of Hosp. for Sick Children, Toronto, Ont., Canada): THE ACTION OF BZ-55 IN DOGS. PART I. OBSERVATIONS ON DEPANCREATIZED HOUSSAY DOGS. Canad. M. A. J. 74:960-62, June 15, 1956.

BZ-55 was found to be effective in the absence of the pancreas and hypophysis. BZ-55 appears to potentiate the action of insulin, since the insulin requirement in the depancreatized dogs diminished markedly. Two depancreatized dogs reacted differently: In one, the insulin requirement could not be changed; in the other, 1 unit every 8 to 10 days replaced 24 units per day during the control period.

Smith, G. W.; and Kumar, D. (Depts. of Med. and OB & Gyn., Univ. of Toronto, Toronto, Ont., Canada): EFFECT OF BZ-55 ON THE INSULIN TOLERANCE CURVE. Canad. M. A. J. 74:997, June 15, 1956.

The authors propose a modification of the insulin tolerance test to try to separate patients sensitive to BZ-55 from those who are not. After being off of long-acting insulin for three days and regular insulin for twelve hours, patients were given 0.1 unit of regular insulin per kilogram of body weight intravenously in the fasting state. Blood sugar level was recorded at the onset and every half hour for a total of three hours. The administration of BZ-55 was then begun; as soon as therapeutic blood level was reached (10 to 20 mg. per cent), a second insulin tolerance test was started. Fifteen patients were so tested with four groups of responses: (1) a considerable fall in the insulin tolerance test after BZ-55 administration; (2) marked fall in the fasting blood sugar with little, if any, change in the insulin tolerance curve after BZ-55; (3) a combination of these; or (4) no response. The authors speculate but draw no conclusions regarding the results.

Spalding, W. B. (Dept. of Med., Univ. of Toronto, and Med. Serv., Toronto Gen'l. Hosp., Toronto, Ont., Canada): CLINICAL RESPONSE TO BZ-55. Canad. M. A. J. 74:992-94, June 15, 1956.

BZ-55 given to two elderly patients with no evidence of endocrine disease produced distinct hypoglycemia. No hypoglycemia was produced in the third patient with hypopituitarism after two days of treatment. Of three diabetic patients treated, one patient who had been on 20 units of protamine zinc insulin responded satisfactorily. Another who had previously received no insulin responded satisfactorily, and a third who had been on insulin for fifteen years responded with only mild hypoglycemic effect.

Spitzer, Judy A.; and Spitzer, John J. (Div. of Labs. and Res., New York State Dept. of Health, Albany, N. Y.): EFFECT OF LIVER ON LIPOLYSIS BY NORMAL AND POST-HEPARIN SERA IN THE RAT. Am. J. Physiol. *185*:18-22, April 1956.

Lipolysis by serums heparinized in vivo and by normal serums was studied after perfusion through isolated rat liver. The rate of clearing of an oil emulsion by serum containing clearing factor was decreased after perfusion through the liver. The extent of clearing was not always affected. Clearing-factor activity was lessened by incubation with heparinase (prepared from rabbit livers). Normal rat serum exhibited consistent lipolytic activity following perfusion through the isolated liver. This activity was not due to clearing factor.

Sprague, Randall G.; and Kilby, Ralph A. (Mayo Clin. and Mayo Foundation, Rochester, Minn.): EVOLUTION OF MODIFIED INSULINS IN THE TREATMENT OF DIABETES MELLITUS, WITH SPECIAL EMPHASIS ON INSULIN-ZINC SUSPENSIONS. Am. J. Med. *19*:925-32, December 1955.

The background for the development and the evolution of the modified insulins now available is reviewed. Observations of one hundred consecutive patients treated with Lente insulin are presented together with comments on the future place of insulin-zinc suspensions in insulin therapy. The authors conclude that although one's first reaction to the introduction of the new insulin-zinc suspensions is likely to emphasize the confusion resulting from the availability of additional insulins, it is not unlikely that the new preparations, by virtue of their freedom from modifying proteins and their probable adaptability to the needs of almost all diabetic patients, may eventually substitute for and replace all of the existing preparations except regular and crystalline insulin.

Stadie, William C. (John Herr Musser Dept. of Research Med., Univ. of Pennsylvania, Philadelphia, Pa.): CURRENT VIEWS ON THE MECHANISMS OF INSULIN ACTION. Am. J. Med. *19*:257-73, August 1955.

The current concepts of the problem of the action of insulin upon intermediary metabolism are summarized. Four theories of the mechanism of insulin action are considered in detail: the permeability of transfer hypothesis of insulin action, the hexokinase theory, the effects of insulin on oxidation phosphorylation, and the

possible action of insulin on oxidative reactions in the Krebs cycle. The pertinent experimental evidence in support of each hypothesis is cited. The author concludes that, on the basis of the available evidence, a decision from the four possibilities of insulin action outlined is impossible now. The role of insulin in the relationships between carbohydrate and fat metabolism, as well as fixation of insulin by tissues, is discussed.

Staub, A.; Sinn, L.; and Behrens, Otto K. (Lilly Res. Labs., Indianapolis, Ind.): PURIFICATION AND CRYSTALLIZATION OF GLUCAGON. J. Biol. Chem. *214*:619-32, June 1955.

The isolation and crystallization of glucagon, the hyperglycemic-glycogenolytic factor of the pancreas, are described. Physical and chemical studies indicate that the crystalline protein is of high purity. The amino acid composition revealed that glucagon is a distinct entity with no apparent relationship to insulin. Histidine is the N-terminal amino acid. Glucagon is considered by the authors to be entirely different from the crystalline hyperglycemic-glycogenolytic substance recently described by Mohnike and Boser. The latter appears to be a nucleoprotein and differs in several respects from glucagon. Crystalline glucagon virtually free of zinc is highly active biologically; its activity was not potentiated by addition of either zinc or insulin.

Sutherland, Earl W.; and Wosilait, Walter D. (Dept. of Pharmacol., Sch. of Med., Western Reserve Univ., Cleveland, Ohio): THE RELATIONSHIP OF EPINEPHRINE AND GLUCAGON TO LIVER PHOSPHORYLASE. I. LIVER PHOSPHORYLASE; PREPARATION AND PROPERTIES. J. Biol. Chem. *218*:459-68, January 1956.

Previous studies from the authors' laboratory showed that the concentration of phosphorylase in liver slices changes rapidly in response to certain experimental conditions. The concentration of phosphorylase in the cells is considered to represent a balance between inactivation of the enzyme and reactivation to the active form. Experiments designed to aid understanding of the mechanisms involved in regulation of the concentration of liver phosphorylase in intact cells are reported in a series of three papers. This paper deals with the preparation of a purified liver phosphorylase.

Tepperman, Jay; Tepperman, Helen M.; and Schulman, Martin P. (Dept. of Pharmacol. and Biochem., State Univ. of New York, Upstate Med. Center, Syracuse, N. Y.): OXIDATION OF PALMITIC ACID-1-C^{14} BY TISSUES OF CARBOHYDRATE AND FAT DIET-ADAPTED RATS. Am. J. Physiol. *184*:80-82, January 1956.

Groups of adult male rats were adapted to isocaloric high-fat and high-carbohydrate diets for 4 to 6 weeks. Liver slices and segments of diaphragm were incubated in a medium to which an emulsion containing palmitic acid-1-C^{14} had been added. Respiratory CO_2 was collected and precipitated as $BaCO_3$, and the specific activity was estimated. Liver slices of fat-diet-adapted rats oxidized more of the added palmitic acid than did those of carbohydrate-fed controls. Diaphragms of the former group also oxidized more added palmitic acid but abstracted less glucose from the medium than did those of the latter.

Tomizawa, Henry H.; and Williams, Robert H. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): STUDIES ON THE SPECIFICITY OF AN INSULIN-INACTIVATING SYSTEM OF THE LIVER. J. Biol. Chem. *217*:685-94, December 1955.

The question of specificity of an insulin-inactivating system of the liver was investigated by the technic of substrate competition, in which the effect of various proteins upon the extent of degradation of insulin-I^{131} was measured. The insulin inactivation system, considered to be probably of a proteolytic nature, was found to have some degree of specificity; however, other substances, such as α-corticotropin, casein, glucagon, and growth hormone, could also be substrates for this system.

Watt, J. G. (Dept. of Med., Univ. of Toronto, and Toronto Western Hosp., Toronto, Ont., Canada): TREATMENT OF UNSTABLE DIABETES MELLITUS WITH BZ-55 AND INSULIN. Canad. M. A. J. 74:983-85, June 15, 1956.

Two patients with labile diabetes were not improved by the addition of BZ-55 to their treatment program. The sulfonamide levels maintained were considerably higher than usual.

Welsh, George W.; Henley, Elaine D.; Williams, Robert H.; and Cox, Robert W. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): INSULIN I^{131} METABOLISM IN MAN: PLASMA-BINDING, DISTRIBUTION AND DEGRADATION. Am. J. Med. *21*:324-38, September 1956.

The authors have undertaken to determine differences in the metabolism and distribution of insulin I^{131} in persons with and without diabetes and to investigate the cause and significance of such differences.

Intravenously injected labeled insulin was retained in the plasma of many insulin-treated persons with diabetes longer than in those without diabetes. Plasma capable of binding insulin was shown to protect against insulin hypoglycemia in mice which suggested that a plasma-binding factor is present in many insulin-treated persons with diabetes.

Their study demonstrated that I^{131}-labeled insulin disappears less rapidly from the plasma of the majority of insulin-treated diabetic subjects and from schizophrenic patients than from the plasma of nondiabetics. Seventy-nine per cent of the diabetic group showed a significant retardation in the rate of disappearance of labeled insulin from the plasma at sixty minutes. Between 15 and 90 per cent of the dose of labeled insulin remained circulating in the plasma. In contrast, the nondiabetic control group retained between 4.5 and 13 per cent of the dose within the plasma at sixty minutes. The presence in the plasma of insulin-treated subjects of a factor which binds labeled insulin, hinders its entry into normal rat tissues, and depresses its enzymatic degradation was demonstrated by in vitro studies with the use of dialyzed labeled insulin and heparinized diabetic or normal plasma and by incubation with pieces of rat diaphragm, liver slices, or liver homogenate. This binding action of diabetic plasma with insulin effectively reduces its hypoglycemic action in mice. The authors suggest that the insulin-binding factor may be an antibody arising secondarily to insulin therapy. They add further supporting evidence to explain the discrepancies among the daily insulin requirements of diabetics which are in excess of those needed in depancreatectomized man as well as to aid in explaining marked insulin resistance among some diabetic patients.

Whitney, John E.; Roberts, Sidney; and Beaver, Elsye L. (Dept. of Physiological Chem., Sch. of Med., Univ. of California Med. Center, Los Angeles, Calif.): INFLUENCE OF PREVIOUS DIET ON HEPATIC UTILIZATION OF GLUCOSE IN VITRO. Am. J. Physiol. *182*:51-53, July 1955.

Investigations have been conducted on the influence of diets high in carbohydrate or fat and subsequent fasting on hepatic utilization of glucose in vitro. Feeding a high-fat diet for two to three months depressed carbon dioxide production and glycogenesis from uniformly labeled glucose in the livers of fed rats. The differences in hepatic glucose oxidation in fat- and carbohydrate-adapted animals persisted 24 to 48 hours after the deprivation of food. Total oxidative metabolism in the liver was not appreciably affected by the caloric content of the preceding diet or by short-term fasting. It is concluded that carbohydrate metabolism in the liver is dependent upon the level of carbohydrate in the diet and that liver tissue from animals fed a high-fat diet preferentially oxidizes some substrate other than glucose.

Wolff, Frederick W.; Stewart, G. A.; Crowley, M. F.; and Bloom, Arnold (Wellcome Foundation and Whittington Hosp., London, England): TRIAL OF AN ORAL HYPOGLYCAEMIC AGENT IN DIABETES. Brit. M. J. *3*: 440-45, Aug. 25, 1956.

The authors studied the response to oral BZ-55 in a group of forty-five diabetics normally requiring insulin. These patients were under conditions of controlled diet. Diabetics in whom the drug was successful often had a history of diminishing insulin requirements. Diabetics in whom the drug failed tended to have a higher insulin requirement than the previous group. BZ-55 does not exert any influence over the postprandial rise in blood sugars, either in the group of successes or in the failures. There is no difference between successes and failures in levels between free and conjugated sulfonamides in the blood. Urinary excretion of conjugated sulfonamides was higher in the failure cases than in the successful ones. Five patients (11 per cent) developed skin reactions due to the drug.

Wrenshall, G. A. (Banting & Best Dept. of Med. Res., Univ. of Toronto, Toronto, Ont., Canada): THE SIGNIFICANCE OF GLUCAGON. Canad. M. A. J. 74:871-74, June 1, 1956.

The author reviews present knowledge and possible clinical usefulness of glucagon.

Wrenshall, G. A.; and Best, C. H. (Banting & Best Dept. of Med. Res., Univ. of Toronto, Toronto, Ont., Canada): EXTRACTABLE INSULIN OF THE PANCREAS AND EFFECTIVENESS OF ORAL HYPOGLYCAEMIC SULFONYLUREAS IN THE TREATMENT OF DIABETES IN MAN—A COMPARISON. Canad. M. A. J. 74:968-72, June 15, 1956.

The authors present analyses of 158 autopsied diabetics in whom pancreatic extractable insulin was determined. Relating results to age of onset and duration of diabetes, they found a basic similarity in pattern between presence or absence of appreciable amounts of extractable insulin and effectiveness or ineffectiveness of either of the two oral sulfonylureas in returning blood sugar levels and sugar excretion levels toward normal.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1956-1957

SUGGESTED MEDICAL STANDARDS FOR CAMPS FOR DIABETIC CHILDREN

Last year the Chairman of the Committee on Camps appointed a Subcommittee on Medical Standards. It was clearly recognized from the outset that no committee of the national organization or its Council had any direct authority, or in many instances any authority at all, over camps for diabetic children, because these camps had developed through individual or group efforts outside the framework of the American Diabetes Association and in many cases not in direct association with an Affiliate. It was felt, however, that suggested standards might be established and sanctioned by the Council for the assistance and guidance of those in charge of established camps or those planning new ones.

It is evident that no standards will fill the needs of all camps. Some camps are mature well-established institutions that own their own land, buildings and equipment and have a sizable paid staff, sometimes with year-round employees; while on the other hand, some are represented by a situation in which a few boys or girls are allowed to attend a camp that primarily is for other purposes in which some special dietary and nursing care is provided.

The Subcommittee on Medical Standards has attempted to set up a skeleton plan along these lines:

1. Entrance Requirements
2. Personnel
3. Medical and Laboratory Equipment
4. Regulations Relating to Sanitation

It is the consensus that little should be said by this Committee regarding the methods used in the management of the diabetes itself in a camp since this must be left to the judgment of the medical personnel in charge.

In order that such a program as is outlined may be as practical as possible most of the sections are considered in two categories: Firstly: What is ideal? And secondly: What should be considered minimal acceptable standards?

The opinions of the members of the Subcommittee varied considerably on many points. For example, some members felt there should be no preliminary entrance requirements, such as physical examination and inoculation programs. Others thought preliminary examination should be done before the child comes to camp and certain immunization regulations should be obligatory. Such

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Report of Subcommittee on Medical Standards of the Committee on Camps, completed Seventeenth Annual Meeting June 1956; reviewed by the Executive Committee per authorization of the Council granted at the 1955-56 Interim Session.

procedures, while possibly ideal, might under the best existing circumstances not be possible—for example, if children were coming from long distances, possibly even from foreign countries.

What follows therefore may not be in every point sanctioned fully by all members, but it is the closest approximation to a consensus which the Subcommittee has been able to reach.

ENTRANCE REQUIREMENTS

IDEAL. 1. Each applicant be examined by his home physician, member of the Camp Committee or camp physician preferably within a week before the child comes to camp. The child should be pronounced free of recent dangerous contact with and free from any contagious disease. If this is not possible, such examination should be done upon arrival and before acceptance at camp.

2. All applicants should have had the usual childhood inoculations, including diphtheria, pertussis and tetanus toxoid, as well as appropriate booster injections.

3. Vaccination against smallpox should have been done within the preceding three years.

4. It is suggested that the child should have received typhoid and paratyphoid inoculations or appropriate booster doses.

5. X ray of the chest should have been taken within one year before coming to camp.

6. A recent record of the diet and insulin dosage should be sent to the Camp Committee or medical authority in charge.

7. Salk vaccine injections should be considered and given if thought advisable by the medical authorities in charge of the camp.

8. The emotional status of the child or his physical defects should be commented upon by the attending physician.

MINIMAL. 1. That the family physician certify that the child is free of any contagious disease or that an adequate screening examination be made on arrival at camp.

2. All applicants should have had the usual childhood inoculations, including diphtheria, pertussis and tetanus toxoid, as well as appropriate booster injections.

3. Vaccination against smallpox should have been done within the preceding three years.

4. It is suggested that the child should have received typhoid and paratyphoid inoculations or appropriate booster doses.

5. That a recent record of the diet and insulin dosage be sent with the child to camp.

PERSONNEL

1. A Camp Committee should be formed to consider and plan the details of medical affairs of the camps and to correlate these with other camp activities.

2. Members of the Camp Committee should be physicians and lay persons interested in diabetes.

3. The Chairman of the Camp Committee should be a physician with experience in, and special knowledge of, diabetes. He should be a member of the Board of Trustees of the camp and might hold other positions such as that of Executive Director or camp physician.

4. A Lay Director should be appointed. He should be properly experienced in all phases of camping and should have or be given an adequate knowledge of diabetes and all such problems as are involved with it in a camp. The Lay Director or a recreational director responsible to him should plan and implement the recreational and various craft programs.

5. The Lay Director of the camp should be subordinate to the Executive Director or Chairman of the Camp Committee.

6. The suggested personnel of the Camp Committee is as follows:

a. Chairman, a physician.
b. Lay director or lay executive director.
c. Other interested lay persons or professional persons who are members of the sponsoring organization.

This Committee should collaborate closely with the staff concerned with the operation of the camp.

7. It is highly desirable that clear lines of authority be decided upon in each camp between the nursing, dietary or other professional personnel and the camp physician on the one hand, and the nonprofessional personnel and the lay director on the other.

8. It is strongly urged that wherever possible, there be a resident physician at camp. This is particularly desirable if the number of campers exceeds about twenty-five at one time. The resident physician should be a graduate physician past the intern stage with some special knowledge of diabetes acceptable to the Camp Committee. If this is not possible, camps should have readily available the services of a nearby practicing physician with some interest in and knowledge of diabetes. It is advisable to have daily visits to the camp by a consulting physician or one of the medical sponsors of the camp if a resident physician is not present.

9. The nursing service should be in charge of registered nurses. It should cover twenty-four-hour duty. If a physician is not available at all times, at least one nurse

on each shift should have sufficient knowledge of hypoglycemia to administer adrenalin hypodermically and be capable of giving glucose intravenously. All nurses should be familiar with the specific acute problems of diabetes, such as the recognition of acidosis or hypoglycemia.

10. The nurses or the laboratory technicians should be responsible for the collecting and testing of urine specimens. The nurse should be responsible for the administration of insulin and, in the absence of a physician, the treatment of hypoglycemia. The nurses should also be responsible for the administration of first-aid procedures under medical supervision. They should also participate in conducting educational lectures to the campers and to their parents.

11. Dietitians and diets. There should be a dietitian in charge who is familiar with the dietetics of diabetes. She should be a member of The American Dietetic Association. When this is not possible, the post should be filled by a nutritionist or nurse with equal qualifications insofar as the practical handling of diabetic diets is concerned. The diets should be nutritionally adequate in all respects. Unless special considerations arise, the diet should be altered little from that previously prescribed by the child's physician. The adoption of certain standard basic diets may be necessary if camp attendance is high. The dietitians should participate in the educational programs for the campers and their parents.

12. Recreational programs. The lay director or a recreational director responsible to him should plan and implement the recreational and various craft programs.

13. Counselors. Counselors should be appointed and be responsible to the lay director. Each counselor should be responsible for not more than ten children. When possible, some counselors should have special training in certain fields, such as swimming, so that campers can be instructed and given credits according to the American Red Cross standards. Similar national standards may be made to apply to such activities as riflery, archery, and others. The counselors should aid not only in the carrying out of the recreational program but also in the activities of the nurses and dietitians whenever required. Counselors should be familiar with hypoglycemia and the problems of scheduling exercise.

14. A report on each child should be sent to the referring physician at the end of the season.

MEDICAL AND LABORATORY EQUIPMENT

1. Adequate first-aid equipment should be maintained at camp in case of emergency.

2. A laboratory should be maintained at camp. It should be equipped for the performance of tests for urinary sugar, acetone and diacetic acid, and blood sugar determinations. A laboratory technician capable of performing these tests might be represented by the resident camp physician, nurse or medical student.

REGULATIONS RELATING TO SANITATION

Sanitary regulations are important in any camp. Requirements may be the same in some basic problems but differ widely in others depending upon the geographic location. In most localities it is prudent if not necessary to carry out programs of sanitation in close cooperation with the local Public Health authorities.

A program of camp sanitation should be developed for each camp by competent medical personnel. The program should include:

1. Responsibility for the examination of food handlers according to local or state regulations.
2. Testing the water supply at appropriate intervals.
3. Testing the water of the swimming pool or lake as frequently as necessary to maintain healthy conditions.
4. Testing sanitary conditions around the camp.
5. Checking the toilets.
6. Mosquito and insect control.
7. Control of poison ivy and poison oak in the camp grounds.
8. Maintaining proper dishwashing and refrigeration equipment.

MINIMAL STANDARDS FOR PERSONNEL, DIETETICS, AND EQUIPMENT

1. A camp committee composed of physicians interested in diabetes and lay persons with a similar interest. The chairman of this committee should be a physician, particularly interested in this field.

2. A competent lay director, program director, and recreational staff.

3. The ready availability of a practicing physician, perhaps in a nearby town or village. He should have some interest in and knowledge of diabetes.

4. At least one registered nurse, capable and fully able to handle the usual acute diabetic problems, with sufficient clinical judgment to know when to ask for help.

5. Facilities for measuring or weighing food. Diets under the supervision of the dietitian, or if she is unavailable, under the supervision of a nurse or someone else adequately trained in practical diabetic dietetics.

6. Facilities for doing at least the qualitative tests for sugar, acetone and diacetic acid in urine, and a technician capable of performing these tests.

7. Adequate first-aid equipment should be maintained.

FIRST-AID EQUIPMENT RECOMMENDED BY THE AMERICAN RED CROSS

1. Sterile gauze squares, 3" x 3".
2. Adhesive bandage compresses, assorted sizes.
3. Triangular bandages.
4. Sterile gauze in packages, assorted sizes.
5. ½", 1", and 2" adhesive tape.
6. 1" and 2" roller gauze bandages.
7. Tourniquet and scissors.
8. 3" splinter forceps.
9. Wire or thin board splints.
10. Sterile castor oil or mineral oil for eyes (preferably in tubes).
11. One dram eye dropper.
12. Eye glass.
13. Stretcher, pillow, and blanket.
14. Burn ointment, Petrolatum or others at physician's discretion.
15. Antiseptic solution on physician's order.
16. Accident report to be filled out by the first-aid attendant for physician to see later.

ADDITIONAL SUGGESTED ITEMS

1. Aromatic spirits of ammonia.
2. Oil of cloves.
3. Magnesium sulfate.
4. Calamine lotion.
5. Sodium bicarbonate.
6. Hot water bottles or chemical heating pads.
7. Antibiotics and chemotherapeutic drugs.
8. Tetanus antitoxin.
9. Tetanus toxoid.

INSURANCE

The Subcommittee has studied insurance for diabetic camps and suggests the following:

Fire and Extended Coverage and owners, landlord and tenant public liability with first aid attached. The extended insurance would cover windstorm, hail, aircraft, vehicles, smoke and explosion. The owners, landlord and tenant public liability would cover damage to persons caused by the negligence of the camp or its operation. The first aid would provide for the cost of immediate care for a person damaged without legal liability.

The Committee also suggests for consideration insurance which would cover refunded fees caused by epidemics or quarantines. Another type of insurance suggested by the Camp Committee for consideration is the Campers Accident and Health Policy which pays up to certain limits per accident and also to certain limits for sickness and death not arising from diabetes. A policy of this type would cover the child going to and from the camp.

SUBCOMMITTEE ON MEDICAL STANDARDS OF THE COMMITTEE ON CAMPS,
American Diabetes Association

SEVENTEENTH ANNUAL MEETING

The next Annual Meeting of the American Diabetes Association will be held in New York City June 1-2, 1957, prior to the Annual Session of the American Medical Association, June 3-7. The Hotel Commodore will serve as headquarters hotel and individual rooms are available for members during our Meeting, as well as during the American Medical Association Session.

An announcement of the meeting together with a hotel reservation card was sent on November 1 to all Association members, who are urged to fill out the reservation card and send it directly to the Hotel Commodore as soon as possible.

SCIENTIFIC PROGRAM

The first Scientific Session will be Saturday afternoon, June 1. The other Scientific Sessions will be held Sunday morning and afternoon, June 2. A copy of the Program will be sent to all members in advance of the Meeting, and the program will also be published in DIABETES.

Physicians and other scientists are invited by Alexander Marble, M.D., Chairman of the Committee on Scientific Programs of the American Diabetes Association, to submit abstracts, not to exceed 300 words in length, of papers which they would like to present at the Scientific Sessions. Those interested are requested to submit before March 8 eleven copies of the abstracts to expedite review by the Committee.

A Joint Meeting will *not* be held with The Endocrine Society this year. However, we have been informed that that Society will schedule its papers on diabetes for Saturday morning, June 1, preceding our opening session that afternoon. American Diabetes Association members are invited to attend that Endocrine Society session without paying a registration fee providing they present the ADA registration badge at the session, which will be held in the Hotel New Yorker.

ADA IDENTIFICATION CARD

As announced previously, an identification card for diabetics, issued by the American Diabetes Association, is now available. Developed by the Committee on Information for Diabetics and bearing the official seal of the Association, the card will fit the average pocket or purse wallet. The price is $.10 each in quantities of one through nine and $.05 each in quantities of ten or more, both prices including handling and shipping. Upon authorization of the Council, a sample card was sent to each Active Member of the Association.

DIABETES WEEK, 1957

The week of November 17-23 will be observed as Diabetes Week. Each year the week which precedes Thanksgiving is the official Diabetes Week as selected by the Council upon recommendation of the Executive Committee. Affiliate Associations and Committees on Detection of County and State Medical Societies are urged to note these dates in making plans for development of Public Education and Detection programs.

1956-57 MEDICAL STUDENT-INTERN ESSAY CONTEST

The fifth Medical Student-Intern Essay Contest, sponsored by the American Diabetes Association, is open to medical students, interns and physicians within two years after their graduation from medical school. Any subject relating to diabetes and basic metabolic problems may be selected.

An award of $250 is offered to the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. This prize is again made possible through the kindness of the St. Louis Diabetes Association. An award of $50 will be given for the best review article or case report. Papers will be judged on the basis of value of the material and method of presentation.

Members of the American Diabetes Association and subscribers to DIABETES are requested to encourage medical students and interns to enter the contest. Entrants should submit the original and one copy of their manuscript (four additional copies would be appreciated). The manuscript should be typewritten and double-spaced, and mailed by April 15, 1957, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

NEW MEMBERS

ACTIVE

The following were elected as of Dec. 1, 1956, and Jan. 1, 1957:

California
Radding, Jerome — Fresno

Florida
Jewett, Jim S. — Coral Gables

Georgia
Murphey, Alex T. — Augusta

Illinois
Mills, Walter W. — Freeport
Presley, Sophie J. — Chicago
Vondrasek, Earl A. — Chicago

Iowa
Spratt, Irving L. — Iowa City

Louisiana
Dingman, Joseph F. — New Orleans

Maryland
Asper, Samuel P., Jr. — Baltimore

Massachusetts
Baldwin, Arthur D. — Wellesley
LeCompte, Philip M. — Jamaica Plain
Stanton, James P. — Brookline

Michigan
Hamil, Brenton M. — Detroit

Minnesota
Traynor, Mack Vincent, Jr. — Rochester

New York
Ardan, Nicholas I., Jr. — Niagara Falls
Fineberg, Seymour K. — New York
Lanman, Ben Marr — New York

Pennsylvania
Byers, Robert O. — Erie
Franklin, Sidney N. — Philadelphia
Lee, Charles T., Jr. — Philadelphia
Marshall, David S. — Pottsville

Other Countries

Belgium
Hoet, Joseph J., Jr. — Louvain

Germany
Oberdisse, Karl — Dusseldorf

Territory of Hawaii
Brown, Charles S. — Honolulu

ASSOCIATE

The following were elected as of Dec. 1, 1956, and Jan. 1, 1957:

New York
Freedman, Louis — New York

Australia
Decker, Neill — Melbourne

NEWS OF AFFILIATE ASSOCIATIONS

The LOS ANGELES DIABETES ASSOCIATION held its fall meeting Nov. 19, 1956, at the Los Angeles County Medical Association Headquarters, 1925 Wilshire Boulevard, Los Angeles. Jerome Conn, M.D., Professor of Medicine and Head of the Division of Experimental Medicine, University of Michigan School of Medicine, spoke on "Recent Advances in the Development of Oral Insulin Preparations."

In observance of Diabetes Week, the Association sponsored its second annual Diabetes Fair November 18. The Fair incorporated detection procedures, exhibits, movies, and lectures for panel discussions. Each visitor was provided with an opportunity to procure a chest minifilm and a sample screening test for glycosuria.

The next meeting of the Los Angeles Diabetes Association will be held in February and is to be a combined meeting with the Los Angeles Society of Internal Medicine.

The NEW JERSEY DIABETES ASSOCIATION (Clinical Society) held a meeting in East Orange on Jan. 30, 1957. The program included the presentation of "Electrolyte Disturbances in Congestive Heart Failure and Diabetes Mellitus," by Patrick H. Corrigan, M.D., Attending Physician, St. Francis Hospital, Trenton, New Jersey.

The Metabolic Section of the Medical Society of New Jersey will present a program on May 1, 1957. Dr. Norman Murray of Summit is this year's chairman, and Dr. William Levison of Newark is secretary. The following papers and authors have so far been selected for presentation and two or three more are planned: "The Liver in Diabetes," by Carroll M. Leevy, M.D., Director of Clinical Investigation, Jersey City Medical Center; "Oral Hypoglycemic Agents," by John F. Rogers, M.D., Pennsylvania Hospital.

NEWS NOTES

NEW HOSPITAL TEACHING CLINIC TO OPEN

Plans are being made for early occupancy of the Hospital Teaching Clinic building being erected by Diabetes Foundation, Inc. The new building is located in Boston just across Pilgrim Road from the New England Deaconess Hospital to which it is connected by tunnel. It is unique in that it is designed for the study, treatment and education of ambulatory patients at reduced expense.

The second floor is to be operated by the Deaconess Hospital for forty patients with diabetes who need no nursing care and who are able to be up and about and assist in their own treatment.

The first floor and basement of the building will be occupied by the Joslin Clinic which will rent space from Diabetes Foundation, Inc. The latter organization will have its own quarters for an administrative office, statistical investigation, follow-up of patients for statistical studies, library and postgraduate training.

Dedication of the building is planned for Sunday, February 10. Among the speakers will be Charles H. Best, M.D., of Toronto, and Howard A. Rusk, M.D., of New York. Elliott P. Joslin, M.D., President of Diabetes Foundation, Inc., will preside at the ceremonies.

$18 MILLION APPROVED FOR RESEARCH AID GRANTS

Advisory councils to the eight branches of National Institutes of Health recently recommended approval of 1,300 grants in aid for medical research, aggregating $18,000,000. Final action is scheduled to be announced soon by Dr. Leroy E. Burney, Surgeon General of the Public Health Service. These fields of research are scheduled for the following awards:

Arthritis and metabolic diseases: $1,248,589 (140 grants); neurology and blindness: $1,599,860 (128 grants); cancer: $4,956,809 (263 grants); dentistry: $375,917 (47 grants); allergy and infectious diseases: $2,100,685 (186 grants); heart: $4,635,929 (309 grants); mental research: $1,209,236 (104 grants); general research: $1,324,305 (119 grants).

PERSONALS

DAVID ADLERSBERG, M.D., New York City, and IRWIN J. PINCUS, M.D., Philadelphia, were Officers of Instruction of a postgraduate course in gastroenterology arranged by the American College of Physicians at the University of Pennsylvania Graduate School of Medicine in Philadelphia Dec. 3-7, 1956.

IRVING GRAEF, M.D., New York City, was honored November 5 as the retiring chairman of the Board of Directors of the National Committee for Resettlement of

Foreign Physicians "for his distinguished efforts in helping foreign physicians re-establish themselves in their profession in the United States." He was presented with a bronze plaque and two volumes of letters of appreciation at a luncheon in his honor at the New York Academy of Medicine. Dr. Graef will remain national vice-chairman of the committee.

W. STANLEY HARTROFT, M.D., PH.D., Chairman of the Department of Pathology, Washington University, St. Louis, Missouri, will speak in a symposium, "Fats in Human Nutrition," to be held March 15 in the Louisiana State University Auditorium, New Orleans, under the sponsorship of the American Medical Association's Council on Foods and Nutrition. Dr. Hartroft's topic will be "Pathologic Lesions Related to Disturbances of Fat and Cholesterol Metabolism in Man."

FRANCIS D. W. LUKENS, M.D., left for the University of Ankara, Turkey, on November 25, to serve as a visiting professor for three months. He is lecturing in the fields of endocrinology, diabetes and metabolism. Dr. Lukens, whose visit was made at the invitation of Prof. Kazim Aras, Dean of the Medical School, will also review methods of medical education in Turkey.

RICHARD P. STETSON, M.D., Boston, will be General Chairman of the 38th Annual Session of The American College of Physicians scheduled for April 8-12, 1957, in Boston. JAMES F. GLEASON, M.D., Atlantic City, has been appointed General Chairman of the 39th Annual Session to be held April 28-May 2, 1958, in Atlantic City, New Jersey.

OBITUARIES

FRANK A. EVANS, M.D., Pittsburgh, died Dec. 13, 1956, at the age of 67. Dr. Evans was born in Allegheny, Pennsylvania, and received his preliminary medical education at Washington and Jefferson College. He then attended Johns Hopkins University and was graduated in 1914. After internship at Johns Hopkins Hospital, he became resident pathologist at the Presbyterian Hospital in New York City. Following military service in World War I, he served as director of the Singer Memorial Laboratory until 1924, when he became affiliated with the Western Pennsylvania Hospital, serving as physician in chief of the medical section until 1955. Dr. Evans limited his practice to internal medicine. A member of the American Diabetes Association since 1941, he was also a member of the American College of Physicians, American Society for Clinical Investigation, and a diplomate of the American Board of Internal Medicine.

DAVID CLEMENTS FRICK, M.D., died in Toledo, Ohio, Nov. 6, 1956, at the age of forty-nine. Dr. Frick, who was born in Toledo, was graduated with the degree of M.D. from St. Louis University School of Medicine in 1933. A member of the American Diabetes Association since 1947, he was also a member of the American Academy of General Practice. Dr. Frick was President of the Toledo Diabetes Association and very active in the management of the Toledo Diabetes League and of Camp Za-ni-ka for diabetic children. He was chief of staff of Riverside Hospital in Toledo, a staff member of Toledo Hospital and St. Charles Hospital, and an associate staff member of Mercy Hospital. A Lieutenant Colonel in World War II, Dr. Frick served four years as a flight surgeon.

CASSIUS HOWARD HOFRICHTER, M.D., was born in 1891 in Cleveland, Ohio, and died recently in Seattle, Washington. After premedical education in Hiram College, Hiram, Ohio, he attended Western Reserve School of Medicine from which he was graduated with an M.D. in 1919. Dr. Hofrichter served his internship and a year of residency in medicine at Cleveland City Hospital. In the 1920's he was one of the early workers with insulin in the Northwest. In 1927 he took leave of absence to take part in a special training course in internal medicine at the University of Vienna. Dr. Hofrichter, whose practice was limited to diagnosis and internal medicine, was on the staffs of the Seattle General Hospital and Children's Orthopedic Hospital. A member of the American Diabetes Association since 1941, he was also a Fellow of the American College of Physicians. Dr. Hofrichter was medical director of the Northern Life Insurance Company.

A VALUABLE LIBRARY

The *Proceedings of the American Diabetes Association,* containing a great body of clinical information of practical use to every physician treating diabetes, may be purchased at a special price of $34.50 for Volumes 2, 3, 4, 6, 7, 8, 9 and 10. Volumes 1 and 5 are out of print. For those who wish to complete their series, separate volumes may be obtained at $5.00 a copy. Please direct your orders to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

The *Proceedings,* previously published annually, was discontinued in 1950 with Volume 10. The publication was superseded by DIABETES, *The Journal of the American Diabetes Association.*

NOW AVAILABLE!

SECOND EDITION

DIABETES GUIDE BOOK FOR THE PHYSICIAN

Copies of the Second Edition of* DIABETES GUIDE BOOK FOR THE PHYSICIAN *are available for distribution.

The Second Edition follows the pattern established by the first (published in 1950) with modifications designed to increase its usefulness to the practicing physician.

The changes include new sample diets for adults and adolescent patients requiring a high caloric intake, low sodium diets, and bland, low-fibre diets, modification of the recommendations for the treatment of diabetic coma, and the addition of a section on the management of diabetes in childhood.

The new edition was prepared by the Committee on Revision of* Diabetes Guide Book for the Physician *of the American Diabetes Association.

The following prices have been established:

1 to 9	**copies,**	**$1.00**	**each**
10 to 24	**copies,**	**$ .95**	**each**
25 to 49	**copies,**	**$ .90**	**each**
50 to 99	**copies,**	**$ .85**	**each**
100 to 249	**copies,**	**$ .80**	**each**
250 or more	**copies,**	**$ .75**	**each**

Send all orders to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y. All prices include handling and shipping.